

New ways with official secrets

The long-awaited proposals for the reform of the British law on official secrecy are better than expected, but leave many details to be hammered out.

PARANOIA is the natural condition of democratic governments, which are perpetually bemused that those who collectively elected them to office are so consistently unappreciative of their defence of the public interest. This misunderstanding of the elected by their electors is nowhere more prevalent than over government policies on secrecy and nowhere more justifiable than in Britain, whose governments have been saddled since 1911 with an Official Secrets Act that is both draconian and so draconian as to be ineffectual. The act consists of only two sections. One declares espionage a crime, and is not controversial; the second makes it a criminal offence for government officials to disclose "unauthorized" official information and for other people to receive the same. Since long before 1972, when a committee under Lord Franks was set up to recommend reform of the act, it has been generally agreed that the act is too broadly drawn; a more practical objection is that governments have learned by experience that juries will not readily convict people of offences under the disputed section. But successive governments have failed to find a formula for reform — until now.

The British government's white paper (policy document) on the subject, published last week, is a good first approximation to enlightened reform. First, the categories of information whose disclosure will be a criminal offence are more narrowly defined than even Franks recommended (in 1972). Second, although the intended legislation will apply criminal sanctions to people other than government officials (such as journalists) who disclose information in the protected categories without authority, the penalties will be decided by the courts, before which the government will usually have to prove that disclosure has damaged the public interest and that the discloser had reasonable cause to believe that it would do so. (For government officials accused of telling unauthorized tales, the onus will be on them to prove they could not reasonably have known that harm would be done.) Third, the white paper specifies, for each of the protected categories of information, tests of when harm has been done that appear to have been sensibly and intelligently drawn. That is the good news.

Provisions

The other side of the coin has two faces, one particular and one general. Many of the details of the proposed bill could turn out to be unduly, even unintentionally, repressive. For example, it is proposed that any disclosure about security and intelligence by a member of the intelligence services should be considered to be harmful; plausible though this may seem, such a stipulation will effectively prevent members of the British intelligence services from making public their organization's involvement in illegal acts. Dr David Owen was right to insist in the House of Commons last week that this provision is acceptable only if there is some means, independent of the government, of monitoring what the intelligence services get up to. It is also proposed that the disclosure of information about British international relations (another protected category) should be considered harmful if it prejudices relations with another government, an interdiction that on the face of things would prevent the publication of news of the British equivalent of the Irangate dealings (if

there were one). These and other anomalies are all the worse because the government intends that the public interest should not be an allowable defence against a criminal charge. And how will international publications such as this (*Nature* is printed at four centres) be affected by the provision that prior publication elsewhere of proscribed information will not be a defence against prosecution? These are matters that will be looked at vigilantly when the bill is published.

Objection

The more general objection to last week's white paper concerns not the proposed reform of the Official Secrets Act, which will at least let people know where they stand, but the general climate of secrecy in Britain. Constitutionally, this derives from the doctrine of the collective responsibility of ministers, giving them sole titular responsibility for authorizing the disclosure of official information, and their immediate officials the opportunity of making partial (in both senses) leaks of it. While there is no reason why reform of the law on secrets should be accompanied by a more open policy on official information outside the protected categories, that is at least as urgent a need (and would make the forthcoming legislation more palatable).

Just how such a policy should be defined is another matter. The view that Britain needs an analogue of the US Freedom of Information Act is constitutionally inappropriate, but experience over the past decade with the working of the act in the United States has not been particularly encouraging. What is needed, in Britain and elsewhere, is a presumption that the government has a duty to answer legitimate enquiries from responsible sources on matters on which it has already made up its mind — and to do so truthfully. The present nudge and wink system by which the background to government policy is explained is as often damaging to the government as to public understanding. One obvious illustration is the now well-known disagreement between the Prime Minister and the Chancellor of the Exchequer on exchange-rate policy; on such a difficult issue, it would not be surprising that two such powerful people should disagree. But in the absence of more than the sketchiest information about the origins of the dispute, the difference of opinion has been dramatized and also trivialized by being represented as a personality clash pure and simple. Who can benefit from that? On a more practical matter, it would not merely satisfy curiosity but be of some public benefit that the basis of the government's decision to stay as a member of the European high-energy physics laboratory (CERN) at Geneva (provided the cost does not exceed £45 million a year — see page 4) should be widely understood: is this the research councils' sober judgement of what they can afford (and, if so, what will they do with the \$10 million a year they will save?), or a political guess at what is practicable? It should not be impossible to devise a published code of conduct that would ensure a flow of legitimately publishable information without embarrassing the British government's legitimate requirement that policy not yet formulated should not be compromised. That (see *Nature* 333, 101; 1988) is how, in Britain, the government might also arrange that its free press acts responsibly. □



What next for space?

The future of the US space programme is still unclear, despite the Space Science Board's report.

THE report of the Space Science Board of the US National Academy of Sciences has been a long time in gestation and, even so, leaves many important issues unresolved (see page 6). The board's diffidence is nevertheless understandable. Its study was originally commissioned by the National Aeronautics and Space Administration (NASA) in 1984, when it seemed that the then future held the prospect of more spacecraft than could be populated by well-designed instruments. In the event, the outlook is not nearly as bright, while the Space Science Board is also plainly in something of a quandary about the extent to which it should bite its sponsor's hand.

1984, it will be recalled, was the year in which NASA, having engineered what appeared to be a successful shuttle craft, won the US administration's support for the construction of a space station. The chances are high that approval would not have been forthcoming if there were not then (as now) signs that the Soviet Union was making much fuller use of men in space than seemed likely in the United States. But what would the space station actually accomplish? To that question, NASA responded traditionally, by asking the National Academy of Sciences. It may not now much matter to NASA that the reply has taken four years rather than two, and that it provides even now an unclear vision of what the space station may do. Is not the project so far down the road that even the next administration is unlikely to call a halt to it?

That, unfortunately for NASA, may be over-complacent. The next president will urgently have to tackle the problem of the US budget deficit, about which bankers overseas recognize that nothing can be done in an election year. It would be an embarrassment, but not an insuperable one, if the United States were to decide to save some part of the \$12,000 million-plus committed to the project. Certainly there is nothing in the report by Dr Thomas M. Donahue and his colleagues that could be misread as a full-throated endorsement of the space station. Remarking on the inescapable need to develop even better automatic instruments than there are at present, and on the uncertainties attending the medical consequences for those who spend very long periods away from terrestrial gravity, the group says it is too soon to tell where the boundaries of the human occupation of space will eventually be drawn. That is hardly what NASA can have been hoping to hear.

Surprisingly, the group is also less than clear about the role of the United States in international collaboration. To be sure, it says that space exploration is an "international activity" and that the United States must be ready to collaborate in all kinds of ways. But then it goes out of its way to insist that collaboration as such should not be the goal, but that scientific objectives should be paramount. Up to a point, that is prudent enough. But might this not have been an occasion when a high-level committee on Donahue lines might have taken up the question of how the corrosive competition in space between the United States and the Soviet Union might in the long run be turned to more fruitful paths? The recommendation that collaborative ventures should not have more than two other major partners "until a record of successful experience accumulates" is unshakeable but hardly what the occasion requires.

But the most glaring weakness of the report is that its intelligent and even imaginative shopping-list for projects that might in future be carried out says nothing about the priority that is attached to them. That is not entirely the committee's fault. The president of the National Academy of Sciences, Dr Frank Press, explains in his covering letter that the board's shopping-list is longer than could probably be accommodated in the foreseeable future, but that the Donahue committee has taken the view that setting priorities would not be appropriate when doing that

accurately would require information from space projects not yet launched. Yet Press was the one who, the other day, insisted that it is better that scientists should rank projects in some order than leave the process to politicians. Might he not have shared his thinking a little more explicitly with the Donahue committee? And what would be wrong with a prescription that the next projects should build on and extend those that have already succeeded? NASA, by contrast, is habitually captivated by the novel, skimping in the process on its support for the analysis of what has already been done. □

Concessions won

British universities have won modifications to the Education Reform Bill, but need extra funds too.

BRITISH universities have succeeded much better than they had any reason to expect in modifying the government's *dirigiste* Education Reform Bill (see page 7). The most important concession by the government so far is the undertaking that the new proposals that academic tenure should generally be abolished will not be allowed to compromise the freedom of individual academics to hold eccentric or unpopular opinions, but in an exceptional all-night sitting last week, the House of Lords also secured a number of concessions on the circumstances in which academics could lose their jobs on the grounds that their fields are no longer relevant to their institutions' needs. Unexpectedly, last week, the government was also defeated on the important issue of whether the Universities Funding Council due to replace the University Grants Committee would make grants to, rather than contracts with, its dependent universities. The government insists that the difference is semantic only, implying that neither word need constrain bureaucratic interference with the way in which British universities are run. But it is far from irrelevant that the issue has been raised.

Much of the reason for these concessions won by the House of Lords is, no doubt, that the government is in a hurry. Its plan is that the machinery for distributing funds to universities should be in place by next April: there is no time for the delay that would follow from an unresolved conflict with the House of Lords — although it will be time enough to be throwing hats in the air after the bill is read there for the last time at the end of this week (7 and 8 July). But the concessions are also a victory for the Committee of Vice-Chancellors and Principals (CVCP), which on this occasion formidably took the initiative in pleading that the bill should be opposed, in showing that it could be opposed effectively and in mobilizing the opposition in the House of Lords. On balance, it has been a good fight well fought.

So does that imply that British universities can now breathe freely? Unfortunately not. There is still no prospect that the funds available from public sources will be anything but constrained. The new funding council will no doubt be compelled to extrapolate into the future the plans on which its predecessor is working to cause most universities to abandon research-led teaching in many fields. Already CVCP has been compelled to point out that the funds so far allocated for the 1990–91 financial year fall short by £50 million of the sum that will then be needed for restructuring — the euphemism for paying off academics made redundant. Yet this prospect coincides with the discovery that the British economy is once more short of skilled people in fields such as electronics and information technology, and with the more general suspicion that a country ever-eager to declare its ambition to compete with the most adventurous national economies (such as Japan) cannot indefinitely limp along with a rate of participation in higher education that is only half as great. The British government has been consistent in its determination not to meet the cost of such an expansion on the traditional pattern, in which case it must finally make up its mind about the means by which it will turn contraction into expansion. That is the issue to which CVCP should now turn its attention. □

US protests about possible drugs for AIDS treatment

■ Concern that treatment is withheld

■ Drugs manufactured only in Japan

Washington & Tokyo

WHEN a group of demonstrators, chained together and wearing black T-shirts bearing the slogan "Silence=Death", poured into the office of a small Japanese company in Manhattan last week, the stunned company staff had at first no idea whether they were about to become the victims of a hijacking or a hoax.

It was a couple of hours before the demonstrators had put across their message. They were protesting that the company's parent organization, Kowa, a textile company based in Nagoya, was refusing to sell a drug called dextran sulphate to US visitors to Japan. Dextran sulphate may — or may not — have some effect against AIDS and is in wide demand for self-treatment by US AIDS patients. It is manufactured only in Japan.

The protesters, who said they repre-

sented the activist group ACTUP (AIDS Coalition To Unleash Power), may not have chosen their target too carefully. Kowa America's business is the import of ladies' shoes and clothing; it does not deal in pharmaceuticals in the United States and the company's executives were not even sure what dextran sulphate was.

But the protest does represent a real sense of concern amongst AIDS patients that new drugs — untested or still under test — that might have some therapeutic value are being withheld.

A week before the Kowa protest, the Food and Drug Administration (FDA) attempted to stop two companies selling AL-721, a lipid-enriched substance that is now taken by thousands of US AIDS patients. A small study conducted by National Cancer Institute scientist Robert Gallo in 1985 showed that AL-721 helped to inhibit invasion of cells by HIV (human immunodeficiency virus). But that was in a cell-culture experiment; there is no good evidence that it has any clinical effect. But there is also no evidence that AL-721 does harm and FDA's attempt to block its sale quickly ran aground. After lawyers from homosexual activist groups stepped in, FDA agreed to allow AL-721 to go on sale provided it was not labelled as a drug.

Dextran sulphate may have more potential as an anti-AIDS drug. A group in the laboratory of National Cancer Institute's Samuel Broder has published evidence that it inhibits virion binding to T lymphocytes (*Science* **240**, 646; 1988). Broder suspects that the drug may also have other anti-viral effects. But he stresses that these encouraging results in tissue culture cannot be extrapolated to the treatment of AIDS patients.

Nobody knows what will happen to the drug when it is orally ingested, nor whether it may have unexpected side-effects.

Although the drug has been in use for 25 years in Japan to treat high blood-lipid levels, Japanese researchers point out that that does not mean it is free from problems. It is dangerous for haemophiliacs to take it and, as most of Japan's AIDS patients are haemophiliacs, there is little interest in testing it as an AIDS drug there. But clinical trials of dextran sulphate are under way in the laboratory of Don Abrams at San Francisco General Hospital. Successes in the laboratory have encouraged a flourishing underground market in dextran sulphate. The drug

comes only from Japan, where it is produced by at least 15 companies, including such giants as Meiji Seika and Takeda Chemical. Although primarily a textile company, Kowa also produces the drug.

According to a Kowa spokesman, dextran sulphate is not usually available in Japan without a prescription. But it has been on sale, in limited quantities, at the 'American Pharmacy', a well-known drug store with many foreign customers, located not far from Tokyo's Ginza central shopping district. A Kowa spokesman said the company will sell the drug directly only to "legitimate" customers and knows nothing of "underground" dealings.

AIDS activist groups stress that there is a case for allowing dextran sulphate's free sale in the United States. Tom Hannon, who helped to found the Community Research Initiative, a New York-based organization that sponsors clinical trials of AIDS treatments, argues that "we know a great deal about the action of dextran sulphate from its 25 years' use in Japan. We also know a lot about its potential from *in vitro* work. People should be allowed to make informed choices of whether they want to take it or not." Community Research Initiative is carrying out clinical trials of AL-721. Dextran sulphate is for sale in New York from People With AIDS, an organization that also markets AL-721. Many thousands of people with AIDS are believed to be self-administering the drug.

So far, FDA has not attempted to interfere with its sale and FDA commissioner Frank Young is generally credited with the view that the FDA should not be an obstacle to the availability of new drugs. But it has the power to stop the sale of dextran sulphate if it wished.

Many AIDS patients groups argue that there should be a general "right to access to unapproved drugs". AIDS researchers, many of whom Hannon characterizes as "arrogant", have argued that clinical trials will become impossible if AIDS sufferers are taking a whole range of underground treatments. But Hannon criticizes clinical trials that are "not looking from the perspective of the patient".

That AL-721 has not attracted the notice of any of the giant pharmaceutical companies is seen by many AIDS patients groups as evidence that the desire for profit dominates AIDS research. AL-721, like dextran sulphate, is already in production and could not now be patented, so big profits could not be made.

Not so, reply the pharmaceutical companies, because patents can be granted for new uses of a drug. And if the possible 'new use' is already widely known, making a patent impossible to grant, then there are always ingenious ways of patenting modified version of the drug. **Alun Anderson & David Swinbanks**

MRC joins hands with Sumitomo

Tokyo

BRITAIN'S Medical Research Council has joined hands with Japan's Sumitomo Corporation to introduce to Japan inventions and drugs developed by the council's research organizations and to establish research links between the council and Japanese corporations.

Under an agreement signed last Thursday (30 June) Sumitomo will act as the council's sole agent in Japan to license technology and drugs to Japanese corporations and to arrange contract and joint research partnerships. The council has already arranged contracts with more than 100 companies in Europe and the United States but none is Japanese.

Christopher Hentschel, director of research programmes at the council's Collaborative Centre who visited Tokyo to sign the agreement, says that the council has a 'shopping list' of things to offer the Japanese but cannot disclose what at this stage. The council was introduced to Sumitomo by the UK company Celltech Ltd which has been represented by Sumitomo in Japan for several years. And the new agreement is expected to lead to an expanded transfer of British biotechnology and medicines to Japan and will also allow for a flow of information in the other direction, according to David Noble, administrative secretary of the council. **David Swinbanks**

French universities finally required to conform to the law

Paris

Now that Lionel Jospin is sure he is Minister for Education in the new French government, following two months of electoral musical chairs, he has promised to end a controversy that has upset universities for the past four years. While an alternative solution is sought, he has given 18 renegade universities until 15 October to conform to a law that has divided the academic community since it was passed in 1984.

In January 1984, Alain Savary finally saw his proposed reforms of procedures for running the universities reach the statute book. This was the first major modification to affect universities since the upheavals of 1968. But, in 1988, 25 of the 74 universities have still not conformed to all the new rules and 18 are opposed to doing so. The 'Savary Law', which changed the composition of governing bodies, the way they should be elected and the procedures for everyday university administration, has been criticized as excessively cumbersome. At recent elections in one Paris university, it took 17 rounds of voting before a new president was eventually named.

Reluctant to make the changes, several universities have counted on inertia and political uncertainty to continue, as before, with the 1968 regulations. In 1986, with a change of government, many felt that the Savary Law would be abolished. When Alain Devaquet, then Education Minister, tried to do just this, he was forced to concede defeat in the face of student riots.

His successor, Jacques Valade, was caught in a compromise when he took over in January 1987. Unwilling to enforce the politically unpalatable Savary Law, yet dissuaded from attempting global reforms, he proposed to accept the *status quo*, with two-thirds of the universities technically outside the law. But Valade tinkered with the Savary Law, reviving a two-tier doctoral system for some disciplines (see *Nature* 332, 388; 1988).

Speaking to a meeting of university presidents on 30 June, Jospin declared this situation untenable. It is the duty of ministers to enforce the law, he said, and the law is that of 1984. If, by 15 October, some universities have still not filed statutes and changed their governing bodies, Jospin has promised to take the matter to court to ensure uniformity "by the end of the year". Jospin has already made the PhD once again the only doctorate, although he has accepted that some disciplines within the arts may interpret thesis requirements "flexibly".

Jospin is not immune to calls to make

the Savary Law easier to apply, but he is unlikely to try to undertake radical changes. He has promised to draw up procedures "in the next few weeks" to simplify the rules, especially concerning the elections of university presidents and of non-academic governors. At the same time, Jospin is aiming to raise university standards and the quality of life for students.

Higher education in France is uniquely heterogeneous. Most engineers (a highly valued title in France), politicians and those with any kind of economic power come up through the elite *grandes écoles*, but these institutions carry out little research. Research is mostly carried out by career researchers, with little or no teaching load, in state-supported centres under the umbrella of organizations such as CNRS and INSERM. Most of these researchers are university graduates. This forces French universities to act as half-way houses, carrying out most of the teaching, but often excluded from the

academic elite.

Jospin would like a better adaptation of university education to vocational requirements. At present only 30–60 per cent (according to disciplines) of first-year students get through their examinations. Jospin wants to see the figures raised to 80–90 per cent by improving coordination between secondary and tertiary education. He has also set up a working party to investigate the future of their discipline at home.

Last month, before President Mitterrand dissolved his new government, Jospin provided money in this financial year to boost library and student residence facilities (see *Nature* 333, 584; 1988) and promised to see the level and number of student grants raised. But, he told French television news last Thursday, students will find the situation after the summer vacation much as Jacques Valade had intended. It is too late to make changes before then, said Jospin.

If the injection of cash and energy into higher education will please students and academics, right-wing deputies in the assembly who, with the 'Centrists', still have political muscle, are already wondering where the money is going to come from.

Peter Coles

UK particle physics looks up as CERN tightens its belt

London

The future of particle physics in Britain is looking up after recent moves towards cutting costs at CERN, the European laboratory for particle physics, which means that Britain is likely to remain a member. The CERN Council has accepted two proposals: to change the formula for calculating each member state's annual contribution to the budget and to reduce the number of staff. The council is also considering plans to open up membership of CERN to countries outside Europe.

The new accounting system could reduce Britain's contribution to CERN by £10 million. That would reduce it from the present £55 million to a level thought to be acceptable to the government. Calculations are at present based on the relative gross domestic products of each country. But the figures are always three to six years out of date.

Britain's lagging economy and the weakness of the pound in the early 1980s have meant that Britain's subscription, in sterling, has increased by more than 35 per cent although CERN's budget has remained steady in real terms. The new formula, likely to be implemented next year, will take more account of exchange rate fluctuations and bring up to date the calculation of gross domestic product.

In line with recommendations of a management review committee chaired by

Anatole Abragam of the French Atomic Energy Commission, the CERN Council has accepted there is a need to reduce staff numbers.

Over the next four years, CERN will shed 200 jobs beyond departures expected from natural wastage. By 1993, CERN will have reduced its staff by 10 per cent to just over 3,000. And by 1995 this will have produced savings of £70 million, a saving of £10 million more than proposed by the Abragam committee.

At the same time as reducing numbers, CERN hopes by recruiting 50 junior staff annually over the next four years to reduce the average age from the present 48 years.

Another cost-cutting move now on the discussion table at CERN is the British proposal to open membership beyond Europe's boundaries. CERN is already an international venture in that researchers from all over the world use the facilities there.

But there is increasing concern that non-member countries do not fully pay their way. They tend to support large projects but not the basic research programme. Under the proposed scheme, such countries would have a form of associate membership and pay full economic costs for access to experiments. A working party is being set up to look into this proposal and is due to report early next year.

Christine McGourty

Japan keeps its options open on genome sequencing project

Tokyo

JAPAN'S role in world-wide efforts to sequence the human genome remains undecided in a new report to the Science and Technology Agency. Perhaps for political reasons, given that the powerful Ministry of Finance has yet to back the project, all possibilities are left open.

The 40-page report, compiled by the Council for Aeronautics, Electronics and Other Advanced Technologies, covers all aspects of human genome analysis, including chromosome sorting, gene mapping and sequencing, automation of analytical procedures, cell banks, data processing and storage, and international collaboration. But, apart from stressing the need for automated analytical techniques and the importance of human genome analysis for diagnosis and treatment of genetic disease, the report gives absolutely no indication of the future course Japan is likely to take.

For example, no indication is given of whether mapping or sequencing of genes should be stressed, although this is a major point of contention among proponents of the human genome project in the West. And the report says it is very important to consider the possibility of setting up one large-scale facility to carry out the analysis. But it then calls for careful examination of whether one or several small facilities should be established. Some academics on the council's biotechnology subcommittee and working group on human genome analysis favour one large facility, whereas some agency officials favour several small facilities.

Surprisingly no mention is made of the technology for automated DNA sequencing that has already been developed in Japan. Professor Akiyoshi Wada of Tokyo University, a member of the sub-

committee and working group, in collaboration with industry and with the backing of the agency has been developing various machines for several years.

Seiko has developed robot systems for base-specific fragmentation of DNA based on the Sanger method; Tosoh (formerly Toya Soda) is developing instruments to pick up specific fragments of chromosomes; Fuji Photo Film Co. has produced a system for mass production of films for gel electrophoresis and autoradiography; Hitachi Software and Seiko have independently developed automatic scanners to read autoradiograph films; Hitachi has a laser system for real-time analysis of DNA sequences on gels marked by fluorescence; and Mitsui Knowledge Industry Co. has software for matching up DNA sequences.

Eiichi Soeda at the Institute of Physical and Chemical Research and colleagues at the institute's life science centre in Tsukuba are trying to combine these machines into one automatic system. And a small committee has been established in the institute, including Wada, to map out

the best route to follow. Wada says that the laser fluorescent method will probably be favoured over autoradiography. At present about 95 per cent of DNA sequencing is carried out by autoradiography, he says, but the trend in Europe and the United States is towards techniques based on fluorescence. The committee has a short-term goal of reading 100,000 bases per day. And Wada is confident this target can be reached in "a year or two". But Wada's ultimate aim is a system that can handle one million bases per day. Much will depend on how much money the Japanese government is prepared to put into the project; for this fiscal year only ¥207 million (about \$1.5 million) has been allocated to the Science and Technology Agency's project, a fraction of the amount being spent in the United States.

The council report makes a vague call for international cooperation. The Science and Technology Agency opened a window of communication with the US Department of Energy last year. But Wada does not expect this window to be used until responsibility for handling the human genome project in the United States has been sorted out among the various US agencies involved.

David Swinbanks

Head of CNRS returns to industry

Paris

SERGE Feneuille, director-general of the largest French science research organization, CNRS, unexpectedly resigned on 23 June. Although Feneuille's departure coincides with a recent change in government and the appointment of a new Minister for Research, he has denied that there is any connection. But, as a friend of the dislodged conservative government, Feneuille's chances of carrying out the radical reforms he was appointed to oversee have been definitively stymied by the socialists' pro-research policy (see *Nature* 333, 584; 1988). Now the tune has changed, he may have felt he was no longer the man for the job.

Feneuille, who comes from industry, was appointed in 1986 by Alain Devaquet, Minister for Research in the then newly elected right-wing government. The CNRS, which conservative critics have long felt is too hierarchical and dominated by left-wing union influence, had already been targeted for reforms before the socialists' last period in office, from 1981 to 1986. But when Devaquet tried to make changes in the CNRS, by dissolving an appointments committee and blocking new recruitments, he started a legal imbroglio which upset the organization for over a year (see *Nature* 327, 647; 1987).

Soon after Feneuille took up his post in June 1986, it became clear that he would

have to wait until the storm died down before reforms could be made. In the meantime, Jacques Valade replaced Devaquet at the ministry and, when the CNRS had almost returned to normal 18 months later, the government changed again.

In the past ten years, the CNRS, with its 25,600 staff and FF8,900 million (£850 million) budget, has had five changes of director. Successive ministers and directors have seen the need for changes but this political hot potato has always been handled gingerly. Ironically, the CNRS annual report was published on the day of Feneuille's resignation. In his preface, written before the recent elections, he makes no secret of the year's turmoil, but says that efforts to "modernize" the organization had been started, without which the CNRS would continue to suffer "burdens of all kinds, running the risk of paralysis beneath often excessive regulations and outdated practices".

Feneuille is now returning to industry, but has promised to continue in office until a successor is found. Although the new research minister, Hubert Curien, shares with his predecessors a desire to open the state research organizations to industry, many feel that the new director will be an academic, like himself. The issue of reforms, which few deny are necessary, will once again be inherited and, perhaps, once again deferred.

Peter Coles

Institute of Molecular Pathology, Vienna

IN our issue of 9 June 1988 (333, 490; 1988), we published an article on the opening of the Institute of Molecular Pathology in Vienna. It has been suggested to us that the juxtaposition of the article and the accompanying cartoon might be read by some as conveying the impression that the institute was concealing from the public the true nature of its activities.

We wish to make it clear that no such impression was intended and we apologize for such distress as may have been caused to the director, Professor Max Birnstiel, and to the staff of the Institute of Molecular Pathology. □

Gorbachev encourages change in science as elsewhere

London

THE Soviet Union "must create a qualitatively new Soviet scientific potential", Mr Mikhail Gorbachev told the nineteenth conference of the Communist Party of the Soviet Union last week. *Perestroika* in science, he said, is not merely a matter of putting right the mistakes and omissions of the Brezhnev era, the "years of stagnation", when Soviet science fell behind in a number of leading fields, and the social status of science and the prestige of scientific work declined rapidly. Without radical changes in the whole approach to science, he said, it will be impossible to achieve a breakthrough in basic research.

Part of the problem, he acknowledged, is economic. "It is abnormal", he said, that only 6.5 per cent of all the funds allocated for scientific research go to the academic sector of science which carries out the bulk of research". But the problem is not only the amount of money but also how it is allocated. He once again stressed the need to set up "scientific and technical complexes" that would override inter-departmental barriers, the development of financial autonomy for research institutions and the encouragement of contract research. Some scientists, he noted with apparent approval, have been discussing the possibility of "cooperative forms" of organizing science, operating in "a sensible combination" with the state sector.

Another problem is what Gorbachev called the "impeding factors" inherited from the past. Centralized "command methods of management" had frequently meant that priorities had been imposed on scientific research "which did not stem from the logic of its own development", while other new and promising areas either received inadequate support or were forbidden altogether. There must be radical changes in the approach of society to science, he said. "When we make requests of scientists, we must show greater trust and create all the necessary conditions for creativity and their search for what is new." In particular, special attention must be paid to research at the interface of different scientific disciplines, as it is in these areas that "revolutionary break-throughs" are being achieved in all branches of science and technology.

Gorbachev's 2,000-strong audience of delegates contained 175 scientists and university teachers and 41 medical personnel, of whom 112 were members or corresponding members of the Academy of Sciences of the USSR, the academies of the union republics or the specialized academies. Many scientists, however, had made known their views and needs well before the conference. Scientists from the

Leningrad institutes of the Academy of Sciences of the USSR which, some time back, combined their resources to form the kind of research centre advocated by Gorbachev accused the presidium of the academy of impeding their work by unnecessary bureaucratic restrictions, in particular by forbidding the Leningrad researchers to earn foreign currency and spend it on improving their own facilities. The president of the academy, Dr Gurii Marchuk, himself a delegate to the conference, told the academy presidium that he advocated limiting the tenure of posts in the academy to two successive five-year terms of office. (Gorbachev had originally proposed such a time limit on state and party officials but the proposal was dropped.)

There were also a number of pre-conference complaints about how delegates were elected — or not elected. Moscow University, in particular, was especially angry when its favoured candi-

date, Professor Popov, described by Moscow radio as "a man well known for his innovative views on *perestroika*" was rejected by the party committee of Moscow's Lenin Hills District in favour of the university's rector and party secretary.

For other scientists, complaints about professional problems were only part of wider issues. In a pre-conference public meeting in Vilnius, the president of the Lithuanian Academy of Sciences, Dr Juras Pozela, stressed that not only was the financing of science in the Lithuanian SSR poor, it was also "unacceptable in principle", as the Lithuanian government was not free to decide how to allot the available funds.

From this he went on to the general question of Moscow's control over the union republics which, he said, he was prepared to raise at the conference. Why, he asked, do the party second secretaries of these republics have to be nominees of the all-union central committee. Lithuania, he said, echoing the growth of national sentiment in the (formerly independent) Baltic republics, is sufficiently mature to manage without such patronage.

Vera Rich

US space sciences look to the future

Washington

STRIKING an upbeat tone, the long-awaited report from the National Research Council's (NRC) space science board on space science in the next century* declares at the outset that the past 25 years have been extraordinarily productive for space science. But the successes have been accomplished in spite of the traditional emphasis at the National Aeronautics and Space Administration (NASA) on putting man into space and accomplishing "large engineering projects for their own sake". The board concludes the time has come to make science and its application to human welfare a central theme of the US programme, alongside manned spaceflight.

The NRC report has had a long gestation. In early 1984, NASA requested a study of the primary scientific issues it would face between 1995 and 2015. The study was essentially complete in late 1986, but the NRC internal review process took more than a year, and it was only in February this year that the board was able to choose a release date. In the end, the board picked 28 June to coincide with its own thirtieth anniversary.

The report consists of seven volumes: an overview plus six volumes on separate scientific topics — astronomy and astrophysics, life sciences, mission to planet Earth, fundamental physics and chemistry, solar and space physics, and planetary and lunar exploration.

Thomas Donahue, chairman of the National Academy of Sciences space science

board for the past six years, says that he hopes the report will be useful as a new White House administration puts together its agenda for space. Donahue is particularly enthusiastic about work done by the task group on fundamental physics and chemistry. Microgravity studies such as the lambda point experiment which measures the heat capacity of liquid helium through its transition from superfluid phase, or potential studies of 'fractal aggregates' — a tenuous form of a space-filling solid — may make possible a new understanding of basic physical science.

But the chief problem facing space science is its constant need to compete with the more resource-hungry manned space programme within NASA. Donahue feels that an independent space science institute, possibly structured along the lines of the National Center for Atmospheric Research in Boulder, Colorado, is an idea worth investigating. He also argues that space science should be budgeted at its full cost so it is not beholden to the space shuttle for access to space.

But Lennard Fisk, associate administrator of NASA and head of the office of space science and applications, does not feel that splitting space science off from NASA would be a good thing. Fisk says it would be too hard to determine precisely where operational programmes end and space science begins to make a separate institute a viable entity.

Joseph Palca

*Space science in the twenty-first century: imperatives for the decades 1995–2015. National Academy Press, Washington, DC, 1988.

Compromise report issued by White House AIDS commission

Washington

THE White House last week released without comment the final report of the Presidential Commission on the HIV [human immunodeficiency virus] Epidemic. The final report recommends a different approach from a draft report released last month (see *Nature* 333, 485; 1988) on the question of how the US federal government should establish effective leadership to counter the AIDS epidemic.

As expected, most commissioners disagreed with the last chapter of the draft report — written by commission chairman James D. Watkins — which recommended declaring the epidemic a national health emergency and setting up Surgeon General C. Everett Koop as an 'AIDS czar' to coordinate the national response.

Instead, the final report contains a compromise recommendation to the

president to appoint a continuing seven-member committee to oversee federal efforts against AIDS, and to establish an independent Department of Health. The report also urges the federal government to streamline procedures for responding to public health crises.

After insisting that it wanted its own evaluation of the AIDS crisis before acting, the Reagan White House does not appear likely to move quickly to implement any of the report's recommendations. Most of the suggested actions will not be paid for in the 1989 budget, and legislation to establish most of the policy changes called for in the report has yet to be introduced. With only six months left in office, the Reagan administration will be able to leave many of the tough decisions about AIDS to the next occupant of the White House. Carol Ezzell

New faces for scientific publishing empire

Washington

THE Institute for Scientific Information (ISI), once totally controlled by president and chief executive officer Eugene Garfield, has formed a partnership with JPT Holdings, a group of publishing entrepreneurs. Although it is not a sale, Paul Neuthaler of JPT Holdings describes his company's financial interest in ISI as "substantial".

Garfield has built the ISI publishing operation on the success of *Current Contents*, the popular weekly listing of contents of scientific publications. In recent years, Garfield has become involved in other ventures that have not been as financially rewarding — ISI Press, the Atlas of Science and the bi-weekly science newspaper, *The Scientist*. Opening a new office in Ireland also put a strain on ISI.

Under the new arrangement, Neuthaler and Joseph Palazzolo will join ISI as executive vice presidents. Garfield will remain as president and chief executive officer. JPT Holdings is headed by Theodore Cross, a veteran of the financial publishing world. He now owns Frost and Sullivan, a market research company and Faulkner & Gray, Inc., publisher of newsletters in law, accounting and banking. Neuthaler says there are no immediate plans to alter ISI's operations, but in the long run the new partners may use their experience to move ISI into new areas such as newsletters and journals.

One problem for the new partners is what to do with *The Scientist*. Described by Garfield as a newspaper "covering the entire spectrum of the business and profession of science", the newspaper has had its share of troubles. Eighteen months after it began publication in October 1986, the editorial offices were moved from Washington to Philadelphia. At the same time, there were major changes in the editorial staff, including Ellis Rubinstein replacing Tabitha Powledge as editor.

As a new publication, *The Scientist* was not expected to start making money for four or five years, and according to those involved with the newspaper it has been losing money as expected. The new partners have approached Verlagsgesellschaft Georg von Holtzbrinck of Stuttgart, West Germany, owners of *Scientific American*, to discuss the future of *The Scientist*. Other options said to be being considered are to maintain *The Scientist* within ISI, but market it more aggressively, or to cease publication altogether. Joseph Palca

Another win for UK academics on reform bill

London

BRITISH academics have won another major victory in their campaign to ward off perceived threats to the independence and autonomy of universities posed by the government's Education Reform Bill, which completed its report stage in the House of Lords last week. Peers rejected plans to change the present grant system and replace it with tighter controls, which academics interpreted as signalling the introduction of contract funding.

In the original form of the bill, the new Universities Funding Council (UFC) was to award to universities "payments, subject to such terms and conditions as they see fit". But in an amendment, those words were replaced by "grants, specifying such particular obligations and subject to such general guidance as they see fit".

Lord Swann, who tabled the amendment, said the government wanted a system that was restrictive, bureaucratic and "hardly trusts anyone to do anything". The Association of University Teachers, which represents over 30,000 academics, said the amendment would temper the potentially wide-ranging powers of the UFC which threatened academic freedom. But the danger is not yet over: ministers may try to reverse the change when the bill goes to the House of Commons next week.

The government has already seen defeat at the hands of the academic lobby in the Lords when it was forced to accept inclusion in the bill of an amendment that gives statutory protection to academic freedom (*Nature* 333, 289; 1988).

Before facing another possible defeat, the government climbed down over its intention to make expensive university staff redundant and replace them with cheaper staff to do the same job. Instead, an academic may be made redundant only if the university abolishes the post.

Academics have welcomed the change. "This is wonderful news", said Professor Mark Richmond, chairman of the universities' Committee of Vice-Chancellors and Principals. "British universities would have been at a serious disadvantage in recruiting academic staff in the world market if this clause had not been altered."

As peers fought for academic freedom, academics at a conference organized by the Council for Science and Society were united in support of freedom for academics to carry out non-commercial research. Professor Martin Rees of the Institute of Astronomy at the University of Cambridge was concerned that there would be a majority of industrialists on the new Universities Funding Council. Christine McGourty

Animal guidelines

London

THE continuing need for cancer researchers to study the behaviour of tumours in live animals has prompted the publication of guidelines* on the welfare of such animals in laboratories. The guidelines are sponsored by the United Kingdom Coordinating Committee on Cancer Research (UKCCCR), and the emphasis is on informed judgement of humane endpoints. The authors are at pains to point out that their suggestions should not be taken as gospel, but can and should be modified in the light of new information and developing research needs. Henry Gee

*UKCCCR guidelines for the welfare of animals in experimental neoplasia. Available from the UKCCCR secretariat, Medical Research Council, 20 Park Crescent, London W1N 4AL.

Access to scientific journals

SIR—Edward G. Goldberg says of this institute (*Nature* 332, 10; 1988): "... This distinguished institution, with more than eighty scientists and a large number of laboratories outside its central facility in Zagreb, has single subscriptions to both *Science* and *Nature*, which are kept under lock and key in the Zagreb Library." A few lines below, referring to "... a superabundance of periodicals from international agencies such as UNESCO, UNEP, FAO and WHO", Goldberg suggests diverting a small fraction of these costs to "... the reproduction and distribution in adequate numbers of articles and reports from *Science* and *Nature* to the laboratories and scientific facilities of countries in the developing world".

I hoped that nobody would read and refer to this letter but sadly I was wrong and we have to deal with the consequences.

Goldberg's letter, in spite of its good intentions, views our institute from a rather skewed angle. Few institutions can become and remain "distinguished" by keeping *Science* and *Nature* under lock and key. The Ruder Bošković Institute (with a staff of 400 scientists, rather than Goldberg's 80) is either not distinguished, or it does not keep these journals under lock and key. In this institute, devoted largely to fundamental research in hard sciences, very few things are under lock and key, least of all journals and books.

The role and costs of the 'grey' literature have been discussed elsewhere. There is hardly a publication, journal or periodical, that can boast 100 per cent scientific or informational value (whatever that means). But many of the guidelines, evaluation reports, 'cookbooks' and critical reviews produced by the various UN agencies have their role in many laboratories and governmental agencies around the world. Consequently these should not be discussed as an either/or item with current periodicals. Nor is the selective copying and distribution of information from *Science*, *Nature* and other journals a viable solution. The problem for many countries and their scientific institutions is lack of foreign exchange. Payment in national currencies has long ago been shown as impractical in the light of market conditions for both scientific societies and publishing houses.

May I suggest another possible way to solve this problem? The World Bank requires proof of the environmental compatibility of every investment project before it approves a loan. Maybe it should also make it mandatory that the receiving country provides proof of allocation of some minimum level of investment moneys to the acquisition of proper open literature. The same should apply to

similar governmental or international bank investment loans. Considering the sums floating in such loans, a small proportion of it would solve all the problems.

VELIMIR PRAVDIC

Ruder Bošković Institute,
POB 1016,
Zagreb, Yugoslavia

SIR—I would like to reassure Edward D. Goldberg (*Nature* 332, 10; 1988) that some organizations are working to remedy the severe lack of scientific journals and books in developing countries.

The Australian Centre for Publications Acquired for Development (ACPAD) was established six years ago with the aim of collecting surplus books and journals in Australia for distribution to tertiary institutions in South-East Asia and the Pacific. Funds are provided by the Australian International Development Assistance Bureau through the International Development Program of Australian Universities and Colleges. This allows ACPAD to meet the costs of sorting and listing, and shipping approximately 3,000 cartons or 75,000 items overseas annually.

Fifty institutions in Indonesia, Thailand, Papua New Guinea, Fiji, Malaysia and the Philippines are now included in the ACPAD programme. Donations of material, which are received from libraries and individuals, are listed and offered overseas, allowing libraries to select titles that will be of use to them. When supplying a backset of a journal, ACPAD attempts to provide subsequent volumes and currently provides 350 serial titles to a number of libraries on a continuous basis.

Many organizations, such as the American Chemical Society and the Netherlands Universities Foundation for International Co-operation (NUFFIC) Periodical Project, have similar schemes. And the International Centre for Theoretical Physics, the Third World Academy of Sciences, the International Council of Scientific Unions and the Association of Geoscientists for International Development are organizing a workshop in October on increasing the flow of scientific literature to Third World institutions.

PRUE WATTERS

Australian Centre for Publications
Acquired for Development (ACPAD),
GPO Box 2006,
Canberra ACT 2601, Australia

Leukaemia in UK

SIR—Simon Hadlington's headline "UK leukaemia study implicates nuclear reprocessing plants" (*Nature* 333, 587; 1988) should surely read "UK leukaemia study acquits nuclear processing plants", the

doses of radiation received from these being far too low to have been responsible.

The large excesses of child leukaemias at Seascale and Thurso clearly call for an explanation other than chance; but the 165 child leukaemias at Gateshead on Tyneside, and several other groups of child leukaemias far from any nuclear plant, surely provide conclusive proof that other causes than radiation can be responsible. No mention was made in Hadlington's article of the fact that raw sewage at Seascale and inadequately treated sewage at Thurso were discharged on their respective beaches, strongly suggesting a local carrier of an infectious agent — which anyway should surely be considered whenever clusters of cases of any disease are found to occur.

A smaller and statistically less striking cluster similarly suggestive of infection occurred in an estate of 100 households north of Slough, where three child leukaemias occurred in two years, two of them to unrelated children living next door to each other.

The unreasonable confinement of serious studies to the neighbourhood of sources of artificially produced radiation must have delayed for five years or more the investigation of alternative possible causes.

J. H. FREMLIN

46 Vernon Road,
Edgbaston,
Birmingham B16 9SH, UK

Leading sentence

SIR—I hesitate to comment on the leading article by John Maddox ("How not to catch attention", *Nature* 332, 391; 1988) for fear of appearing to support scientific writing that wastes words with ineffectual banalities.

The lead sentence in a scientific article, however, may be far less important in drawing the readers' attention than are lead sentences in other types of literature or news reporting. For example, what attracts me to a scientific article is the title; if it appears to be of interest, I then read the abstract. A poorly written, uninformative or confusing abstract will generally lead me to skip the rest of the article. If the abstract encourages me to read on, I then look at the figures and references for a quick evaluation of whether the material is new and up to date. Only after passing this last check will an article reach the 'to be read' stack. Unfortunately, by the time I have a chance to get back to the article, I'm usually in such a rush that I just skip the first few paragraphs of the paper and look for the 'meaty parts' instead.

WILLIAM R. NORMARK

US Geological Survey,
Branch of Pacific Marine Geology,
3475 Deer Creek Road,
Palo Alto, California 94304, USA

Jumping the greenhouse gun

People who should know better have begun to talk as if they know the greenhouse effect has begun to work its way through the climatic system. That is premature.

ARE the effects of the expected greenhouse effect already with us? The view that they are has recently been given more attention than it should have been in evidence to the US Congress, and by Dr Kenneth Hare, the chairman of the climate planning board of Canada. Simple members of the US Congress, mindful as always of their constituents' interests and conscious of the severe drought that has brought many farming operations to a halt during this growing season, have been looking for a simple answer with which to respond to a common plight. What the Congress, and the rest of us, must understand is that it will never be possible to answer affirmatively the question "Is this the year the greenhouse effect began to bite?" The best that can be hoped for is that it will be possible retrospectively to note that this or that climatic effect is probably a consequence of this or that driving force.

This state of affairs is unavoidable, but not unprecedented. Indeed, this is precisely the kind of trouble people repeatedly encounter in, for example, trying to decide whether the decline of some species is a consequence of loss of habitat or of a more direct insult, such as a change in the pattern of pesticide use. Even recognizing that the greenhouse effect has arrived is bound to be among the more difficult exercises in relating effects to causes because there are so many conflicting influences and because of the complexity of the observational situation.

That is why it is worth remarking that, even in the past few decades, the history of the greenhouse problem is far from simple. One of the earliest systematic attempts to guess at the consequences of accumulating atmospheric carbon dioxide was the massive study carried out at the Massachusetts Institute of Technology in 1960, which predicted warming of the average surface temperature of the Earth by about 1° C by the time that carbon dioxide had doubled from its supposedly pre-industrial condition. That study, unfortunately, coincided with a period during which climatologists, concerned at the cooling trend beginning in about 1940, were more concerned to utter warnings of the difficulties that would be occasioned by the return of a general glaciation.

In reality, of course, there is no inconsistency between that alarm and Hare's assertion that global temperatures have been increasing for the past century, and

have by now increased by a total of 0.4° C. It is entirely possible that a downward fluctuation may be accommodated within a generally rising statistical time series. Indeed, there is no reason why the cooling trend from 1940 for a quarter of a century may not have been consistent with increased retention near the surface of the Earth of energy inputs from the Sun; the accumulation of excess thermal energy may have been more than compensated for by its transfer to the heat reservoir of the global oceans. All that is easily understood, but suggests that there will be no sure way of telling that carbon dioxide has begun to do its expected work on periods of time shorter than those on which contrary effects have been recognized.

The problem, of course, is the familiar one of signal and noise. The larger the signal relative to the noise, the more easily it will be recognized. The greenhouse signal, however serious its long-term consequences, is in the short run relatively small. The interannual variations of average surface temperature at one particular spot on the surface of the Earth are comparable with the changes expected from a further doubling of carbon dioxide, so that the owner of a single thermometer, however sensitive, cannot hope to tell which way the trend is pointing. But it is also well known that attempts to calculate changes of average global temperature from regional measurements of temperature are complicated by the disparity of the quality of observations from one region to another on the surface of the Earth.

The best hope is that Earth satellites, by concentrating attention on the parameters that really matter (in this case, the balance between the inward flux of solar energy and the outward flux of infrared energy), will be able to provide a much less noisy background against which to look for a signal. Increasing temperature may be the parameter to fear, but it is not the one efficiently to watch. Hopes that it may be possible to find climatic characteristics, the frequency or strength of prevailing winds for example, that will be at once more sensitive and more reliable indicators of climatic change, seem similarly doomed to failure. Other things being equal, one would expect a parameter sensitive to change to be one subject to unusually great fluctuation. And while it may be possible to glean something worthwhile from, say, the retreat of glaciers and ice-caps where they persist, the inertia of these effects is inevitably so

great that awaiting a clear signal from the noise is certain to mean waiting until it is too late.

The exercise might be a little simpler if there were not such good reason to believe that the link between carbon dioxide (and the other greenhouse gases) and climatic change is shot through with uncertainty. Although it has been known for nearly three decades that the quantity of carbon dioxide lodging in the atmosphere is only half of that discharged into it, whether the missing half finishes up in the biosphere or in the oceans is unknown — but is critical for the long-term prognosis. The link between an accumulation of excess heat and the surface temperature is similarly, but seriously, complicated by uncertainty about the role of the oceans as heat reservoirs.

These, unfortunately, are only some of the uncertainties. The well-known weakness of the climatic models, in this connection, is that real clouds (as distinct from average cloudiness) are on the face of it a source of negative feedback that may substantially moderate the expected size of the increase in temperature of the surface of the Earth. The variability of solar output is another spanner more recently thrown in the works. Is it a secular change, random or linked with the solar cycle? The disconcerting recognition that chlorofluorohydrocarbons, the chief candidates of ozone destruction, are likely to be more efficient moderators of solar energy is, however, unequivocal bad news.

What in the circumstances should people do? Sell farmland in the Middle West, putting the money into Canada instead? Move away from coastal cities against the threatened increase of the height of sea-level? At this stage, that would be precipitate. One year's drought does not make a greenhouse. For the time being, there are only two sensible courses of action that might be taken. First, urge that public funds be put into the direct measurement of the energy balance of the Earth; as things are, there is a danger that researchers and their sponsors will give themselves too great a sense of comfort by spending time and effort on work not directly related to what needs finding out. Second, urge that governments should prepare themselves for deciding what to do when (not if) the greenhouse effect becomes palpable. The increase of surface temperature might be reversed by not burning fossil fuels but not the melting of the ice.

John Maddox

Supramolecular chemistry

Unnatural product synthesis

Fraser Stoddart

DESPITE the fact that over six million organic compounds are known, it is only now that a closed molecular container compound (Fig. 1) has been fashioned¹, by Nobel laureate Donald Cram and his group at UCLA. The so-called carcerand (from the Latin *carcer*, meaning prison) is large enough to imprison behind its covalent bars chemical small fry, like molecules of the solvents benzene, ether and chloroform, ions such as caesium (Cs^+) and chloride (Cl^-) or an argon (Ar) atom. Up to six water molecules can be accommodated together: they are just small enough to squeeze in and out of the small portals at the ends of this first molecular cell, shaped like an American football. As well as having aesthetic appeal, this unusual molecule could be a prototype of future drug-delivery compounds and other useful products.

In his Nobel lecture², Jean-Marie Lehn states that "chemistry is not limited to systems similar to those found in biology, but is free to create novel species and invent processes". The techniques of molecular engineering and design combine to make possible the synthesis of diverse, useful and novel compounds. Unnatural product synthesis is largely concerned with making compounds with commercially desirable physical or biological properties and with syntheses intended to test structural hypotheses or to establish chemical reaction pathways. But many chemists also design compounds simply for their appealing molecular symmetry — an activity that focuses on the esoteric aspects of chemistry. Pedersen's crown ethers³, Lehn's cryptands⁴, Cram's spherands⁵ and their derivatives^{1,6}, all organic 'host' compounds that bind to smaller 'guests', exemplify this field of designer chemistry.

Cram's new carcerand¹ is the first rigid container compound to be synthesized.

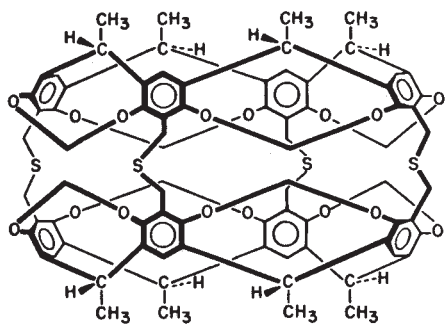


Fig. 1 Cram's carcerand¹ $\text{C}_{80}\text{H}_{72}\text{O}_{16}\text{S}_4$, shaped like an American football. Four carbon-sulphur bonds (middle) form during the last step of its synthesis to hold the two 'cavitand' precursors together. (Courtesy of D. J. Cram.)

Because of its virtual insolubility in any medium, the carcerand is very difficult to purify. This practical problem is a result both of its intractable properties and the mode of its synthesis from two hemispherical 'cavitand' precursors — a tetra-thiol with four attacking thiolate groups ($-\text{S}^-$) and a tetrachloride with four chlorine atoms ($-\text{Cl}$) capable of departing as Cl^- ions. The critical shell-closing step involves the making of four carbon-sulphur bonds under an atmosphere of argon in a concoction of two solvents, tetrahydrofuran $[(\text{CH}_2)_4\text{O}]$ and dimethyl formamide $[(\text{CH}_3)_2\text{NCHO}]$ containing

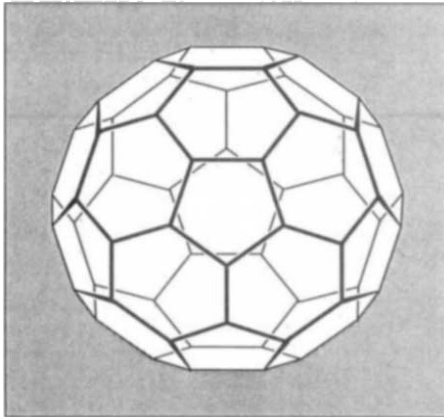


Fig. 2 Buckminsterfullerene⁷ (footballe), a C_{60} species composed of 12 pentagonal rings and 20 hexagonal rings in the shape of a pentagonal dodecahedron.

pulverized Cs_2CO_3 . As they form, the carcerand molecules trap a range of chemical bits and pieces present in the surrounding broth. The observed molecular formula of these complexes of carcerands, which are termed carcaplexes, is $\text{C}_{83.84}\text{H}_{77.99}\text{O}_{21.18}\text{S}_{4.039}\text{Cl}_{0.35}\text{Cs}_{0.35}\text{Ar}_{0.0065}$, interpretable in terms of the average chemical picture $\text{C}_{80}\text{H}_{72}\text{O}_{16}\text{S}_4$ (the carcerand) + $0.39[(\text{CH}_3)_2\text{NCHO}] + 0.67[(\text{CH}_2)_4\text{O}] + 0.35(\text{CsCl}) + 0.0065\text{Ar}$ (the guests) + $\text{O}_{4.12}$, where only the last four or so atoms of oxygen are unaccounted for.

The carcaplexes are more than just molecular garbage cans: because of their high thermal stability, they could be used as molecular ball-bearings. Attachment of metabolism-resistant carcerands, containing radiation-emitting guests, to immunoproteins could be used to hunt down and kill cancer cells. Very large biodegradable carcerands could be used as slow-release drug-delivery systems and could extend the lives of agrochemicals, such as insect pheromones and tickicides employed in sheep dips. Speculation aside, the significance of the research is that a new class of organic host has emerged from the molecu-

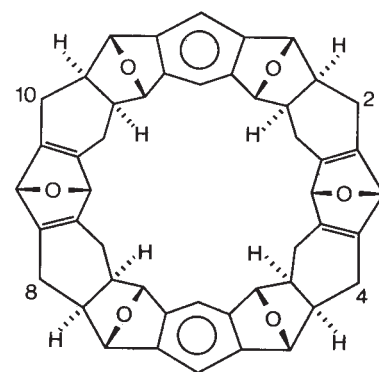


Fig. 3 Kohnke, $\text{C}_{48}\text{H}_{40}\text{O}_6$, formed from readily available precursors by successive Diels-Alder reactions that proceed with high stereo-electronic control.

lar drawing board as a chemical reality.

As Cram⁵ sees it, "the opportunities for fruitful research in the host-guest complexation field are boundless". The quest for unnatural hosts respects no classical chemical boundaries. Topologically akin to the American football (Fig. 1) is the soccerball-like inorganic C_{60} compound (Fig. 2) dubbed buckminsterfullerene, or footballe, formed when graphite is vaporized by laser irradiation⁷. Although this carbon cage⁸ awaits rational synthesis and authentication¹, its calculated electronic stability⁸ (per carbon atom, it is almost twice as stable as benzene) and the recognition⁹ that the pentagonal dodecahedron is, after the helix, nature's second favourite structure, suggest that buckminsterfullerene could be synthesized by standard processes. One of my colleagues (Franz Kohnke) has synthesized¹⁰ a belt-like organic compound (Fig. 3) using the Diels-Alder reaction to elaborate the four cyclohexene rings at 2, 4, 8, and 10 o'clock in the molecule. This synthetic approach, which is highly selective in a stereochemical sense for the isomer shown in

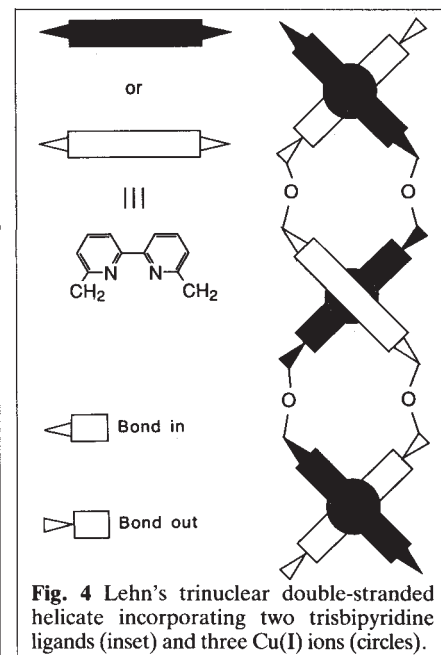


Fig. 4 Lehn's trinuclear double-stranded helicate incorporating two trisbipyridine ligands (inset) and three $\text{Cu}(\text{I})$ ions (circles).

Fig. 3, could be adapted to build cage compounds including buckminsterfullerene.

Lehn and co-workers⁶ use spontaneous assembly as the guiding mechanism to form a metallo-organic double helix (Fig. 4), similar to the double-helical structure of DNA. When copper (I) cations are added to a chloroform solution of a tris(bipyridine) ligand, double-stranded helicates with two ligand molecules wrapped around three tetrahedrally coordinated Cu(I) ions are produced. Moreover, there is experimental evidence of positive cooperativity — complexation of one metal ion facilitates binding of the next and so on. Also, when ligands containing different numbers of bipyridine units are allowed to compete for the metal ions, pairing occurs preferentially between the same ligands, so that self-recognition must operate. Other organic ligands, containing more and different metal coordinating units, and additional metal ions possessing a variety of first and second sphere¹¹ coordination characteristics, await investigation. For biologists, this offers a way of designing novel poly-

metallic complexes with the potential to form multiple bonds with DNA, thus opening the way to thermal and light-induced strand cleavage with special selectivity features. Polyelectron exchange and photoinduced charge separation are both feasible if multicentre metal (Cu(I), Ru(II), Re(I), ...) complexes can be strung together. The dynamic structural characteristics of self assembly, cooperativity, and self-recognition make these new double-stranded helicates, together with their physical properties, attractive for the design of molecular electronic devices. □

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Fraser Stoddart is in the Department of Chemistry, Sheffield University, Sheffield S3 7HF, UK.

Cell motility

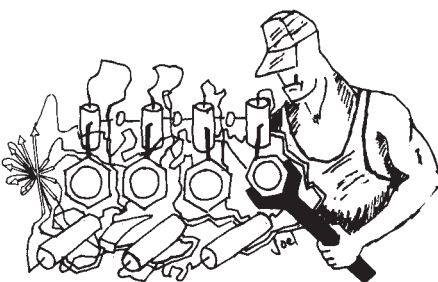
Molecular motor mechanics

Malcolm Irving

A MUSCLE only a few millimetres in diameter can generate a force of a few newtons, which would be enough to allow you to pick up this week's copy of *Nature*. In each basic structural unit along the muscle (a half-sarcomere), there would be about 10^{12} molecules of the motor protein myosin, each exerting a force of about 1 piconewton (pN). Until a few years ago, studies of the mechanism of muscle force generation were made on large populations of myosin molecules, typically about 10^9 acting in parallel; the long extrapolation to the molecular mechanism left many basic questions unanswered. On page 74 of this issue¹, Kishino and Yanagida report force measurements on a population of only 10–100 myosin molecules. With recent developments in quantitative light microscopy, it should soon be possible to resolve the force or motion produced by a single myosin, allowing direct investigation of its molecular mechanism.

This remarkable technical advance follows the introduction of *in vitro* movement assays for motor proteins, and in particular the system developed by Yanagida *et al.*² and by Kron and Spudis³. In this system, myosin molecules are bound to a flat surface. A solution of fluorescently labelled filaments of actin, the protein against which myosin pulls *in vivo*, is applied. The actin filaments bind to the myosin and, when ATP is added, they can be seen to slide over the surface.

In the study reported in this issue, Kishino and Yanagida go a stage further and attach the actin filaments to flexible glass microneedles, ingeniously using inactivated myosin as a glue. If one of the tethered actin filaments is then captured by the myosin-coated surface, a force is exerted on the filament, bending the



needle. Direct measurement of needle deflection in the light microscope shows that a force of about 10 pN is produced per micrometre of actin filament in contact with the surface.

How many myosins pull on each micrometre of actin filament? Kishino and Yanagida estimate the density of myosin molecules on the surface from the ATPase activity. They then calculate that each myosin exerts a force of about 0.4 pN, assuming that all the myosins lying within range of an actin filament can interact with it. This is likely to be an underestimate, as some of the myosins may not be lying in the optimal orientation. So the results

from measurements on populations of 10–100 myosins give about the same estimate of molecular force as that based on measurements on whole muscles: about 1 pN per myosin molecule.

Each myosin is actually composed of two globular heads attached to a long α -helical coiled-coil tail from which the backbone of the myosin filaments in muscle is built. Each globular head contains an actin- and an ATP-binding site and, when isolated by proteolysis, the heads can themselves cause movement of fluorescent actin filaments *in vitro*⁴. But the force required to move an untethered actin filament is much smaller than that exerted by a myosin molecule in a muscle, so this observation left open the question of whether myosin heads can generate substantial forces in the absence of the rest of the molecule. Kishino and Yanagida now answer the question¹ by measuring the force produced by small numbers of isolated head fragments. They find that the force per head is similar in isolated heads and in intact myosin. Thus it seems that the entire motor machinery necessary for generating both force and motion is contained in each myosin head.

The stage is now set for measurements of both the force and the motion produced by single motor molecules. Gelles *et al.*⁵ recently showed that the position of a microscopic bead can be determined with a precision of a few nanometres in the light microscope. These beads were being driven along a single microtubule by a few molecules of another motor protein, kinesin. If the image-analysis techniques of Gelles *et al.* are combined with the mechanical measurements of Kishino and Yanagida, the result is an apparatus capable of measuring both sub-piconewton forces and sub-10-nm displacements, which should allow the action of single motor molecules to be resolved.

Such experiments should answer some basic questions about the mechanism of force generation. For example, how far along an actin filament can a myosin travel for each ATP hydrolysed? Do myosins 'row' themselves along the actin filament in a cycle of attachment to and detachment from it, as is usually assumed? A simple mechanism of this kind would predict that the total force produced by a small population of myosin molecules would fluctuate as individual molecules attach or detach. Mechanical measurements on single myofibrils, in which the properties of a population of about 10^6 myosins are monitored, have already provided some evidence that the force fluctuations are smaller than predicted by this simple mechanism⁶. High-resolution force measurements on smaller populations should provide a critical test of such models. These developments are analogous to the evolution of methods for the study of ion channels in membranes, from

100 years ago

PERSONAL IDENTIFICATION AND DESCRIPTION



The thumb-mark has been used . . . in preventing personation, and in putting an end to disputes about the authenticity of deeds.

The question arises whether fingermarks remain unaltered throughout the life of the same person. I am enabled to submit a most interesting piece of evidence, which thus far is unique, through the kindness of Sir Wm. Herschel. It consists of the imprints of the fingers of his own hand, made in 1860 (left) and in 1888; that is, at periods separated by twenty-eight years. There is an obvious amount of wearing and of coarseness in the latter, but the main features in both are the same.

From *Nature* 38, 201; 28 June 1888.

whole-cell recording through noise analysis to single-channel recording. The analogy illustrates the enormous potential of techniques which allow functional studies of single protein molecules.

The new approach to molecular mechanics is not limited to the study of myosin. On the contrary, the microneedle method of force measurement was used some years ago to estimate the force on a single moving chromosome in anaphase⁷ (about 50 pN per kinetochore microtubule) and the force responsible for microtubules sliding in flagella⁸ (about 1 pN per dynein arm). Any motor protein which is driven by ATP hydrolysis is limited to a maximum energy input of 50 kJ mole⁻¹, or about 10⁻¹⁹ J per ATP hydrolysed. If its efficiency were, say, 10 per cent, it could exert a force of 1 pN over a distance of 10 nm. It has already been shown that myosin and dynein operate in this range of force and displacement, and it seems likely on thermodynamic grounds that kinesin and other ATP-driven motor proteins will also do so. The new high-resolution methods have great potential for investigating these mechanisms of biological motility at the molecular level. □

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Malcolm Irving is in the Department of Biophysics, Cell and Molecular Biology at King's College London, 26–29 Drury Lane, London WC2B 5RL, UK.

Photosynthesis

Roofs and ceilings of light-response curves

D.S. Bendall and J.C. Gray

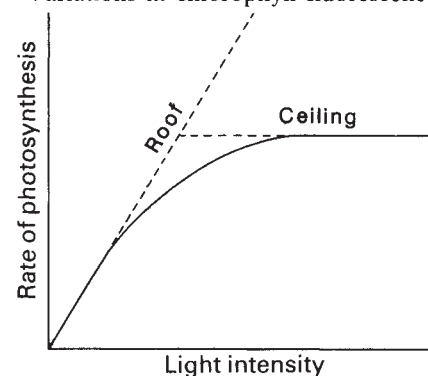
PHOTOSYNTHESIS, a highly complex metabolic system, can adapt to a wide range of environmental conditions. But it is only half the story. Textbooks usually neglect to mention the almost equally important process of photodissipation—the controlled conversion of excess light energy into heat. This is surprising because almost everyone is aware of the great instability of chlorophyll exposed to light, once it has been removed from the organized structure of the chloroplast. New insights into some of the many control mechanisms involved in photosynthesis and photodissipation were the subject of two recent, associated meetings*.

The classical description of photosynthesis is due to Blackman¹ and the central concept that of limiting factors. At low light intensities, for example, the limiting factor is the rate of absorption of photons (see figure). The rate of CO₂ fixation is proportional to intensity and the slope of the line (the 'roof' of Rabinowitch²) is a measure of the quantum efficiency. The rate increases linearly until other factors, such as temperature or CO₂ concentration, become limiting and determine a maximum rate or 'ceiling'—the underlying limitation is the catalytic capacity of the dark reactions of CO₂ assimilation.

The development of a computerized leaf electrode by D.A. Walker (University of Sheffield) has so simplified the determination of light curves that our knowledge of the varied responses of leaves to light under different physiological conditions is likely to be transformed. The intersection region of the roof and ceiling is particularly important. Biochemists brought up with Michaelis-Menten kinetics have difficulty in accepting that a true description might involve a discontinuity rather than a gradual transition from an initial slope to a ceiling approached asymptotically. Walker suggests that the most meaningful measure of the curvature is the area underneath the curve expressed as a percentage of the area under the ideal Blackman roof and ceiling. The value of this 'light-utilization capacity' depends on the sharpness of response of the control mechanisms as the chloroplast adjusts to photodissipation; in practice it can be well over 90 per cent. As shown by S.P. Long (University of

Essex), the observation of low values is often the result of self-shading, of the lower half of a leaf by the upper, for example. The point was made most dramatically at the second meeting, when T. Vogelmann (University of Wyoming) reported that with the aid of glass fibres of diameter 1–5 µm inserted into a leaf, the chloroplasts in upper layers of cells could be seen to filter out nearly all the red and blue of the incident light.

Variations in chlorophyll fluorescence



Experimental (solid line) and ideal Blackman (dashed lines) light response curves (based on Fig. 26.6 of ref. 2).

provide a powerful method for probing energy flows in photosystem 2 because fluorescence is in competition with photochemistry (photochemical quenching, q_o) and acts as an indicator of non-radiative dissipative mechanisms (energy-dependent quenching, q_e). The latter process comes into play in the transition from roof to ceiling. E. Weis (University of Düsseldorf) proposed a model for q_e in which protonation of reaction centres by the low pH of the thylakoid lumen converts them to an energy-dissipating form which generates heat with high efficiency. This raises the intriguing possibility that this process involves a reduced pheophytin molecule of the photochemically inactive arm of the reaction-centre dimer.

P. Horton (University of Sheffield) put forward an alternative model (based on the observation that antimycin specifically inhibits q_e with little effect on the intrathylakoid pH) in which energy dissipation is the result of wasteful cycling of electrons via cytochrome *b*-559. Not only would this provide a function for the cytochrome, which is closely associated with the photosynthetic reaction centre, but it also raises a further question about the nature of the switch (could this be a function of one of the extrinsic regulatory polypeptides associated with the water oxidation

*New Vistas in Measurement of Photosynthesis, The Royal Society, London, 25–26 May 1988; and Physical and Biological Techniques in Photosynthesis Research, CIBA Foundation, London, 27 May 1988.

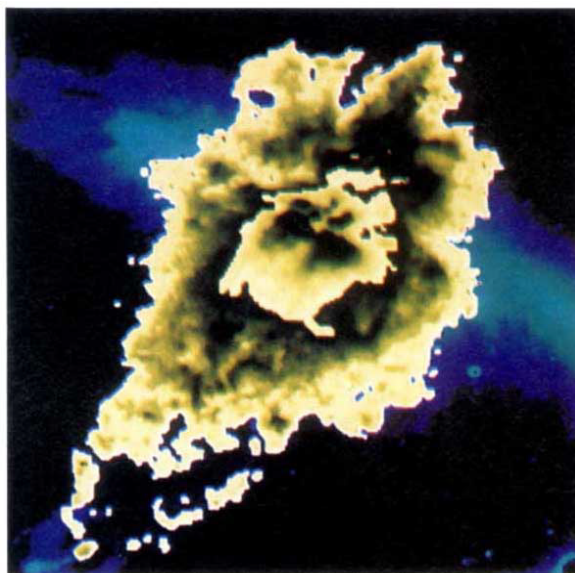
system?). Whatever the eventual outcome of this debate, the physiological significance of such dissipative mechanisms cannot be fully understood without parallel measurements on photosystem 1, neglected until recently because of its failure to fluoresce significantly at normal temperatures. In Weis's model, dissipation in photosystem 1 is the result of efficient internal conversion by the oxidized form of P700 which accumulates at high light intensities. U. Schreiber (University of Würzburg) has developed an instrument for use with leaves which takes advantage of the absorption band of P700⁺ at 820 nm, where little else absorbs.

Roofs and ceilings are inadequate to describe the regulation of the carbon-fixation pathway. Under light-limiting conditions these reactions are not simply coasting downhill limited only by substrate supply, as shown, for example, by the fact that as the light intensity and overall rate increase the ratio NADPH/NADP⁺ decreases (U. Heber, University of Würzburg). The theory of metabolic control developed by Kacser and Burns³ rejects the old idea of a single rate-limiting step and allows the quantitative description of the distribution of control among several steps. This approach has been applied by M. Stitt (University of Bayreuth) to a study of carbon flux and metabolite levels in mutant *Clarkia* plants with different amounts of plastid and cytosol phosphoglucose isomerase (PGI). PGI operates near to equilibrium and has been conspicuously absent from most previous discussions of regulation. Stitt showed, however, that the control coefficient is about 0.1; decreased amounts of plastid PGI result in decreased CO₂ fixation and starch synthesis, but a slight increase in sucrose synthesis, whereas decreased amounts of cytosolic PGI results in increased flux to starch but only small decreases in sucrose synthesis. A detailed analysis of elasticity coefficients of reactions involved in sucrose synthesis shows how the chief contribution to control tends to shift from fructose-1,6-bisphosphatase to sucrose phosphate synthetase as the light intensity is increased. The importance of these observations is that they point the way to a quantitative analysis of the control of carbon flux. The possibility of producing transgenic plants containing altered levels of individual enzymes promises to revolutionize our understanding of photosynthetic control. □

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D.S. Bendall is in the Department of Biochemistry and J.C. Gray is in the Department of Botany at the University of Cambridge, Cambridge CB2 1QW, UK.

The irregular galaxy M82 has puzzled astronomers since Lynds and Sandage postulated in 1963 that an enormous explosion had occurred in its nucleus a million years ago. The figure, a false colour version of Fig. 1 in J. Bland and R.B. Tully's paper elsewhere in this issue (*Nature* 334, 43–45; 1988) shows the emission intensity of hydrogen- α emission in M82. Bland and Tully have looked at the outflowing gas using Fabry–Perot spectrometry, a technique in which the emission at each pixel in the two-dimensional image is



resolved into a spectrum. The wavelength of the H α line is red- or blue-shifted according to its motion within the galaxy. Fabry–Perot observations thus yield a three-dimensional picture of M82, the third axis representing the velocity of hydrogen along the line of sight. Bland and Tully find two distinct components with different velocities, a faint halo rotating with the galaxy and a brighter network of filaments moving out from the centre of the galaxy. This is strong evidence for a 'galactic wind' of outflowing gas, powered by successive supernovae in the galactic nucleus. □

Evolution

Friends and relations

Henry Gee

THE adage that fate makes our relations but choice our friends seems curiously reversed in contemporary systematic theory, where one's circle of friends is determined by one's choice of (evolutionary) relations. In traditional fossil-based amniote phylogenies, birds are closely allied with crocodiles whereas mammals are the last in a long line of synapsid 'reptiles' traceable to the beginnings of the amniote radiation. This assumption has been challenged by cladistic theorists^{1,2} and defended by a proponent of the traditional view³. Now, Jacques Gauthier and others⁴ have assessed all the evidence and come up with some surprises about the role of fossils in phylogenetic reconstruction. They conclude that the use of fossils is permissible, but that some fossils are more useful than others.

On the premise that fossils are not informative enough to overturn a phylogeny based exclusively on data from extant taxa, Gardiner¹ and Løvtrup² used neontological data exclusively to show that birds and mammals are more closely related to each other than to other amniote groups (*a* in the figure). Gardiner tried to show that birds and mammals share so many derived characters exclusively that the likelihood of their not being immediate cousins is remote. He unites them in a clade called Haemothermia, a

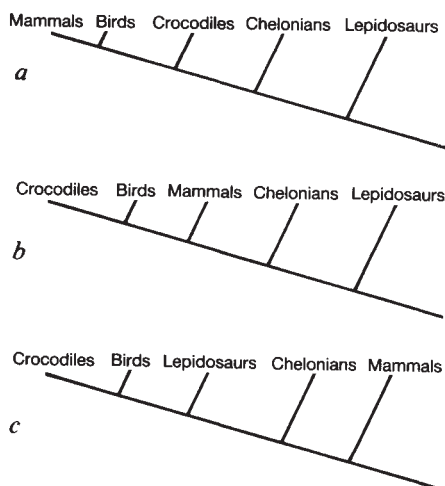
term coined in the nineteenth century.

Kemp counters³ that the fossil record tells a clear, and very different, tale. Far from mammals being related to birds, the transition between primitive reptiles and mammals through a long sequence of progressively more advanced intermediates is backed up by good fossil and stratigraphic evidence. Indeed, the clear organizational trends from stem reptiles through pelycosaurs, cynodonts, tritylodontids, morganucodontids and finally eutherians are beyond dispute, whether one sees them as truly phylogenetic³ or simply an imposition of a time element on a series of grade groups¹. In a cladogram, these trends are broken down into nested sets of character states. Taxa nearer the tips have a relatively greater proportion of derived characters than those nearer the bottom.

Gauthier *et al.*⁴ now reanalyse Gardiner's and Løvtrup's data to discover whether fossils are so uninformative that their use in phylogenetic reconstruction is never justified. Although recasting Gardiner's data gives a very similar cladogram (*b* in the figure), the hypothesis of Gauthier *et al.*, based on 5 living and 24 fossil groups of amniotes, resembles the traditional view (*c* in the figure). Thus, the cladist proposition is overturned: the effect of fossil data on a neontologically based analysis is drastic. Gauthier *et al.*

find that the presence or absence of mammal-like reptiles is crucial to the way the phylogeny turns out. When they are deleted from the algorithm, mammals come to rest among the birds and crocodiles. When they are included, the traditional view prevails. But to say that mammal-like reptiles tip the balance is rather coarse.

Gauthier *et al.* ask whether the fulcrum rests in any particular place along the line between pelycosaurs and primitive mammals. Testing individual mammal-like reptile taxa in turn, they find that the pivot lies not at either end of the reptile-to-mammal chain, but in the middle.



Friends and relations among the main amniote clades. *a*, Gardiner's cladogram based on analysis of the distribution of 37 shared derived characters of extant taxa. *b*, Cladogram of extant taxa from ref. 4. Note that mammals and crocodiles are switched with respect to Gardiner's cladogram. *c*, The diagram from ref. 4 after adding fossils produces a traditional tree, but the discovery that some fossils are more important than others is crucial. Extant groups only are shown for easy comparison with *a*.

Removing all mammal-like reptiles except for either a primitive taxon such as *Casea* or an advanced nearly-mammal morganucodontid could not break up the mammal-archosaur pairing. Including taxa slightly further in from either end (the pelycosaur *Ophiacodon* or a tritylodontid) gives equivocal results. Inclusion of any mammal-like reptile in the great middle ground in between, from advanced pelycosaurs, through a succession of synapsids with progressively more derived features, tips the balance away from Gardiner and Løvtrup and towards Kemp.

These intermediate animals are pivotal precisely because they are intermediate, each one a mixture of primitive amniote and derived mammalian features. The conclusion reached by Gauthier *et al.* is that adding such a fossil taxon to a cladogram based on Recent taxa pulls mammals away from all other amniote taxa, because of the derived features shared by mammal and synapsid. The

relative primitiveness of these synapsids compared with mammals is a result of their having less time to evolve, and not a consequence of evolutionary reversals. The synapsid-mammal group branches off near the root of the tree because of the primitive (or more generalized) features of the synapsids.

Critics argue that any attempt to order Recent and fossil taxa in one grand scheme based on character states is flawed, because character states are relative. The polarity of a character in an animal can be assessed only with respect to the state of the same character in another animal. Furthermore, to assign a character as intermediate between two others excludes the possibility of its being an autapomorphy in its own right. Time distorts the picture, because the stratigraphic age of a fossil generally correlates with primitiveness — a fossil animal is relatively primitive because it has not had time to evolve into something more derived. This makes

good empirical sense, as judged from the fossil record, but poses problems for theorists wishing to expunge evolutionary circularity from their arguments.

As well as the supposed irrelevance of fossils, this is a reason why some cladists prefer to look at extant taxa only; they still exist and yield more data than fossils, and also they are all on the same time plane. When Gauthier *et al.* use data from Latest Triassic through Recent amniotes only, they produce a cladogram similar to Gardiner's. Nevertheless, the new work is a valuable advance, because it not only elucidates amniote relationships but also raises questions about the methods used to reconstruct them. Continuing arguments about both topics will keep the fur and feathers flying. □

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Henry Gee is on the editorial staff of Nature.

Meteorites

Chondrites and the solar nebula

Jeffrey N. Grossman

CHONDRITES are the oldest objects known on Earth. These meteorites are composed mostly of particles that formed in the early solar nebula and also contain rare grains that solidified before the formation of the Solar System¹. The several types of chondrites, broadly classified as carbonaceous, ordinary and enstatite, formed in different locations in the nebula, possibly at different distances from the Sun. A few chondrites, including the Felix (CO) carbonaceous chondrite discussed by Misawa and Nakamura on page 47 of this issue², remain relatively unchanged since their aggregation 4.5 billion (10^9) years ago. Misawa and Nakamura use this to place important constraints on the temperature evolution of the solar nebula as components of the chondrites formed.

Chemical, petrological and isotope studies of chondrite particles, including chondrules and calcium-aluminium-rich inclusions (CAIs), reveal that they formed in many conditions of temperature, oxygen fugacity and cooling rate from heterogeneous solids that existed in the nebula. These particles are the only direct physical evidence of events that can otherwise be observed and modelled only by astronomers and astrophysicists. One of the most important problems is to deduce how particles with different inferred histories were related to each other in time and space in the nebula. Misawa and Nakamura² have found a chemical signature common to chondrules and CAIs that may indicate a closer link between these two types than previously known.

Most chondrules are round particles composed of silicate materials. They probably formed when dust aggregates or mineral grains were melted during transient high-energy events in the solar nebula. The nature of these events is not known, although dozens of causes including lightning, frictional heating, impact melting and heat pulses from the early Sun have been proposed. Chemical studies of chondrules, including the present study², have been of little help in understanding the chondrule-heating mechanism. Instead, they give information about the chemical and physical state of matter in the solar nebula at the time of chondrule formation.

The precursors of chondrules, the earlier generation of nebular solids, are not preserved in their original form in chondrites. In some chondrites, chondrule formation has been so efficient that almost all of the precursors were melted. In other chondrites, a substantial amount of dust accreted with the chondrules to form a dark matrix; but in no chondrite is the matrix formed from the same dust as the chondrules. Thus, the only way to learn about the materials present in the nebula before chondrule formation is to study the chondrules themselves.

Studies of the composition, mineral chemistry and textures of chondrules from most of the chondrite groups by myself and others show that several kinds of precursor solids were present in each region of the nebula^{3–6}. The ambient conditions at the time of chondrule formation in each

region were similar: temperatures were low before the heating events (below 700 K) and chondrules cooled quickly after formation. The data of Misawa and Nakamura² contribute to a better characterization of the precursor material of chondrules which contained refractory lithophile elements. The earlier authors cited above concluded that an epoch of condensation or fractional condensation of hot gases in the nebula produced an assortment of refractory and other dust before the chondrules formed. The refractory components of most chondrules contain elements such as rare-earth elements (REE), calcium, aluminium and less-refractory elements such as magnesium, nearly in their solar proportions.

CAIs are less abundant than chondrules and are much richer in refractory elements. Although some CAIs seem to have been melted, many do not. The origins of the many types of CAIs are even more controversial than those of chondrules. Some, like chondrules, are thought to be the products of high-temperature processing of pre-existing solids in the nebula. Others could be the direct products of condensation. Isotope evidence indicates that many CAIs incorporate presolar components not found in chondrules. The conditions that many CAIs formed under seem to have been different from those of chondrule formation: cooling rates were slower, and there is little evidence of low ambient temperatures before their formation.

Several types of CAIs have extraordinary fractionated abundance patterns of REE and other refractory elements. The abundances of refractories can be uniform or monotonic functions of elemental volatility or even hump-shaped functions of volatility as in the group-II CAIs discussed by Misawa and Nakamura. The only processes that can produce Group-II patterns are fractional condensation or evaporation at very high temperatures. Most chondrules previously studied, and presumably the others in Misawa and Nakamura's study, have REE abundances that are uniform or vary smoothly with ionic radius, not with volatility as in group-II patterns.

Misawa and Nakamura now report in this issue² that some chondrules do have group-II fractionated REE and refractory element patterns similar to those previously found only in CAIs. In fact, chondrules with fractionated REE and other refractory elements had been found previously, but were, for the most part, ignored because they were so different from other chondrules. Chondrules nearly identical in composition and texture with Misawa and Nakamura's Felix-8 were found in Ornans (CO) and Allende (CV)^{5,6}. It now seems likely that several per cent of all chondrules in carbonaceous chondrites have fractionated REE pat-

Did the prey predate the parasite?

OVER evolutionary time it is not altogether a rare event for prey to survive being eaten. Some may pass through the predator's digestive system unharmed, others might profit from the gut environment and reproduce there, while in extreme cases the prey might find a new niche after passing through the gut wall. These are probably the most usual evolutionary routes from a free-living to a parasitic lifestyle, but a recent study on the ciliate *Lambornella clarki* reveals a novel twist to the evolution of parasitism among prey species (Washburn, J.O. *et al. Science* 240, 1193; 1988).

L. clarki is often found in pools in tree-holes where it feeds on bacteria and other microorganisms. Occasionally, larvae of the mosquito *Aedes sierrensis* develop in the same treeholes where they prey upon the free-living ciliates. All is not lost, however, if the ciliates get sufficient advance warning of the presence of a potential predator. Shortly after detecting the mosquito larvae, the ciliate population undergoes a dramatic transformation. The potential prey are able to transform into very effective parasites: the free-living pear-shaped ciliates divide and their offspring transform into spherical cells termed theronts. The theronts make small holes in the cuticle of the mosquito larvae through which they enter the haemocoel. Once ensconced within the body of their would-be predators, the theronts are able to absorb sufficient nutrients to reproduce and ultimately kill their hosts. As they die, the parasitized mosquito larvae release numerous free living ciliates, some of which may transform into parasitic theronts and attack surviving mosquito larvae.

The carefully controlled experiments of Washburn *et al.* reveal that water previously conditioned by the presence of mosquito larvae is sufficient to produce a high level of parasite production. Transformation starts after about 40 hours which is, significantly, just short of the time taken for non-feeding first-instar mosquito larvae to develop into efficient predators. The rate of transformation seems not to depend on whether the

terns. In ordinary and enstatite chondrites, which contain far fewer CAIs, no samples with group-II REE patterns have been found among several hundred chondrules studied.

These observations imply that at least some CAIs formed earlier than chondrules, but in the same or a nearby nebular location. Although the origin of CAIs, including those with group-II patterns, is disputed, the chondrules with group-II patterns must have been formed by the melting of an assemblage of grains including lower-temperature components and an already-formed group-II refractory component. As Misawa and Nakamura show², very high-temperature processing

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Above, three free-living trophonts and one parasite theront of *L. clarki*. Below, invasive cysts formed by theronts on the cuticle of *A. sierrensis* larva.

mosquito larvae had been feeding on a ciliate population.

Nevertheless, under controlled conditions, water that has not been conditioned by mosquito larvae also results in the production of a few potentially parasitic theronts. In the wild, such theronts would die within 24 hours if mosquito larvae did not appear. But if they did, the theronts would be at a marked selective advantage over their free-living conspecifics. Unfortunately, it is not yet clear whether the parasitic or free-living ciliate is the ancestral morph. It is not uncommon for the parasitic ciliates to exterminate mosquito populations from naturally occurring treeholes. Whatever further work is done on this fascinating system, the identification of a potential biological control agent for one species of mosquito perhaps holds hope for others.

Paul H. Harvey & Anne E. Keymer

Paul H. Harvey and Anne E. Keymer are in the Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK.

must have occurred before chondrule melting. Perhaps small CAIs occasionally mixed with more normal, lower-temperature chondrule precursors. If so, models of the solar nebula must allow for a gradual change in conditions from those inferred for CAIs to those inferred for chondrules.

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Jeffrey N. Grossman is at the US Geological Survey, Reston, Virginia 22092, USA.

Phototransduction

A role for calcium in adaptation

Edward Pugh and Jennifer Altman

IN 1985, the hypothesis that cyclic (c)GMP is the internal messenger of phototransduction was greatly boosted by the discovery of Fesenko *et al.*¹ of a cation conductance gated directly by cGMP in patches of the plasma membrane of rod cells in the retina. For almost 15 years calcium had been the odds-on favourite for carrying the message of photoexcitation between the disk and plasma membranes². The finding of Fesenko *et al.*¹, however, led quickly to convincing proof that the light- and cGMP-gated conductances are one and the same cation channel and to general acceptance of the 'cGMP cascade' as the biochemical basis of phototransduction³⁻⁵. Nonetheless, problems remain. The cGMP cascade does not account satisfactorily for the relatively rapid restoration of the photoreceptor to its physiological baseline after a flash of light or for the phenomena of light adaptation. Nor does it explain many of the observations originally taken to support the Ca^{2+} -messenger hypothesis or the results with Ca^{2+} chelators used to refute it⁶. Four new reports⁷⁻¹⁰, three of them in this issue, now point to an elegant resolution of these problems, which re-establishes the internal calcium concentration, $[\text{Ca}]_i$, as a key factor in phototransduction and provides the missing link between the mechanisms of excitation, restoration and adaptation.

In a rod receptor in the dark, channels in the outer-segment plasma membrane are held open by cGMP, allowing both Na^+ and Ca^{2+} to flow into the cell and keeping the membrane depolarized; an Na/Ca exchanger in the outer segment, and an Na/K exchanger in the inner segment, maintain the ionic balance (Fig. 1a). Phototransduction starts when a photon is captured by a rhodopsin molecule in a disk membrane. This triggers the cGMP cascade of biochemical reactions (Fig. 2) that results in hydrolysis of cGMP, whose lowered concentration causes the channels to close and the membrane to hyperpolarize as cation inflow is blocked. Na/Ca exchange continues during the light response, so that $[\text{Ca}]_i$ declines¹¹. Several workers have postulated that the decline in $[\text{Ca}]_i$ acts as a negative feedback, accelerating one or more of the restorative reactions of the cascade (Fig. 2) and possibly serving as a signal for adaptation.

Matthews *et al.* on page 67 of this issue⁷ and Nakatani and Yau⁸ on page 69 now provide evidence that $[\text{Ca}]_i$ is responsible for setting sensitivity during light adaptation (Fig. 1b, c). Both groups use the

strategy of trapping calcium in the outer segments of salamander photoreceptors. They do this by removing external Ca^{2+} and replacing external Na^+ with guanidium, which can permeate the cGMP channels so that current still flows through them but Na/Ca exchange is halted. Under these conditions, the response to a step increase of light does not exhibit the sag or recovery phase characteristic of light adaptation (Fig. 1c): both rods and cones behave as if frozen in a state of adaptation that is determined by the amount of trapped calcium⁷ and the response to a light step seems to consist purely of the superposition of unadapted single-photon responses⁸.

What is the biochemical mechanism by which $[\text{Ca}]_i$ sets sensitivity and alters recovery speed? Recent physiological work by Hodgkin and Nunn¹⁰ strongly implicates the enzyme guanylate cyclase, which catalyses synthesis of cGMP, as the site of regulation by $[\text{Ca}]_i$. These authors 'jumped' salamander rods at various times after a light flash into Ringer's solutions in which Na^+ had been replaced with Li^+ to block Na/Ca exchange, or to which IBMX had been added to inhibit cGMP phosphodiesterase (see Fig. 2). The experiments reveal a relatively long-enduring light-activated phosphodiesterase activity, nearly matched by a strongly accelerated

guanylate cyclase activity during the restoration phase of the flash response. The time-course of the accelerated activity is consistent with regulation by the decrease in $[\text{Ca}]_i$.

A problem with guanylate cyclase as the site of regulation by changes in $[\text{Ca}]_i$ is that biochemical evidence has been equivocal about whether $[\text{Ca}]_i$ in the physiological (sub-micromolar) range can effectively regulate the enzyme^{12,13}. The work of Koch and Stryer reported on page 64 of this issue⁹, which argues for the existence of a novel Ca^{2+} -dependent modulator protein, could resolve this problem. These authors find guanylate cyclase from bovine rod outer segments is highly sensitive to variation in calcium concentrations below 0.5 μM . The highly cooperative dependence on $[\text{Ca}]_i$ has a half-maximal point of 70 nM and Hill coefficient $N=4$, suggesting that Ca^{2+} inhibits guanylate cyclase by complexing with a modulator that activates cyclase when no calcium is bound to it. The modulator substance seems to be a soluble protein but is not calmodulin⁹.

These observations pave the way for a complete and comprehensive theory of phototransduction (Fig. 2), encompassing both excitation and adaptation — complete in the sense that no other biochemical entities need be postulated, and comprehensive, because a single set of reactions describing the interactions of these entities could account for all the main phenomena of phototransduction in vertebrates. Conceptually, excitation (filled arrows in Fig. 2) comprises the processes that lead to closure of the cGMP channel. Each excitation step is matched

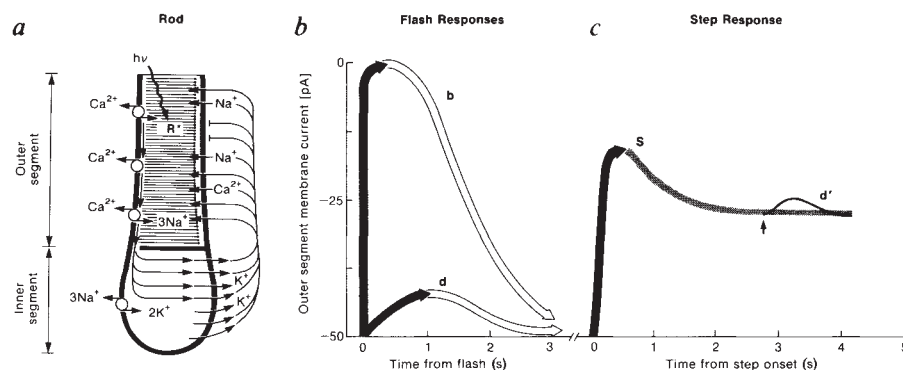


Fig. 1 a, Diagram of a salamander rod showing dark current: in the outer segment ~ 50 – 80 pA of current, carried by Na^+ and Ca^{2+} , flows inward down the concentration gradients. The dark steady-state is maintained primarily by the outward leak of K^+ and ATP-driven Na/K exchange in the inner segment; $[\text{Ca}^{2+}]_i$ is maintained by an Na/Ca pump in the outer-segment membrane. A single photon $h\nu$ isomerizes one molecule of rhodopsin in a disk membrane in the outer segment to become an enzyme (R^*), initiating a cascade of reactions that blocks the inward membrane current of the outer segment. b, Photocurrents produced by brief flashes delivered at time $t=0$ in a completely dark-adapted amphibian rod. The photocurrent is the suppression of the inward membrane current of the outer segment (see a). A bright flash, b, completely suppresses the inward current; a dimmer flash, d, produces an S-shaped curve. Responses to dim flashes increase linearly with light intensity, because each photon suppresses the current in a different compartment in the outer-segment membrane. c, Two ways of characterizing light-adaptation. With a steady light the dark current gradually recovers to a stable level above zero. The recovery phase or sag, s, reveals a feedback mechanism that reduces the efficacy of the incoming photons. When the same dim flash as in b is delivered during the stable phase, the amplitude of the response d' is reduced and its time course decreased. Filled and open arrows indicate the activation and restoration processes, respectively; in reality both occur simultaneously, except very soon after an isomerization.

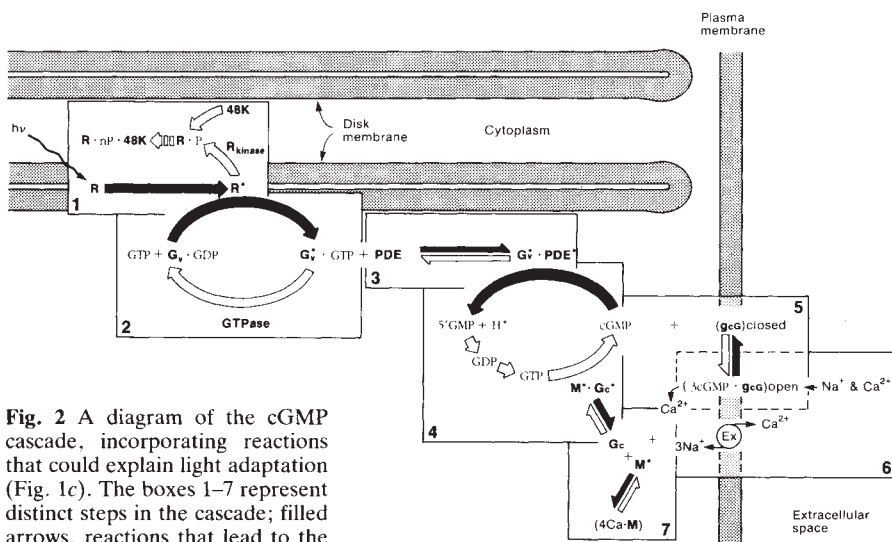


Fig. 2 A diagram of the cGMP cascade, incorporating reactions that could explain light adaptation (Fig. 1c). The boxes 1–7 represent distinct steps in the cascade; filled arrows, reactions that lead to the closure of the cGMP-gated channel g_{gc} ; open arrows, reactions that lead to recovery from excitation and to reopening g_{gc} . Arched arrows, catalysed reactions; straight arrows, binding reactions. 1, A photon isomerizes a rhodopsin molecule R ; 2, photolysed rhodopsin R^* catalyses the exchange of GTP for GDP on the rod G-protein G_v ; 3, activating the cGMP phosphodiesterase (PDE). 4, PDE produces a local drop in cytoplasmic [cGMP], leading to 5, closure of g_{gc} , which 6, blocks entry of both Na^+ and Ca^{2+} into the outer segment. The continued activity of an Na/Ca pump Ex causes a local decline in $[Ca^{2+}]$, which 7, tilts a highly cooperative binding reaction between Ca^{2+} and a modulator protein M to the unbound state M^* , which activates guanylate cyclase G_c to restore the open state of g_{gc} . Reactions 1–3 are accompanied by specific reactions that inactivate the photoactivated protein.

by one or more restoration steps (open arrows) that restore the system to baseline. One of these steps, the guanylate cyclase production of cGMP, is regulated by $[Ca]$, which, because $[Ca]$ declines during excitation, serves as a variable-gain feedback that controls the speed of baseline restoration. Light adaptation is nothing more or less than the accelerated restoration that occurs when $[Ca]$ is lowered by a flash or a steady light.

Cone photoreceptors recover to baseline after a flash much more rapidly than rods of the same species given the same degree of excitation (defined as fraction of initially open cGMP channels closed at response peak). Similarly, cones adapt much faster to steady lights than rods. A satisfying feature of a phototransduction theory incorporating the new evidence is that the well-established morphological differences between rods and cones may account in part for their physiological differences. Cone outer segments are typically smaller in volume than rods but, because their disks form the plasma membrane, have greater surface area. They should therefore be able to lower $[Ca]$ more rapidly when cGMP channels are closed, resulting in relatively faster activation of guanylate cyclase and baseline recovery.

It may seem a paradox that the key biochemical evidence⁹ for the mechanism of $[Ca]$ action comes from mammalian rods, which do not exhibit the speed and sensitivity regulation characteristic of light adaptation in the photoreceptors of lower vertebrates. A reasonable speculation is that cell morphology and body

temperature account for the physiological differences between mammals and lower vertebrates. Single photon absorptions in most mammalian, amphibian and reptilian rods cause the complete suppression of the inward current over a 'compartment' of 1–5 per cent of the length of the outer segment (Fig. 1a). The mammalian rod works at a higher temperature and, because its diameter is smaller (about 2 μm compared with 6–12 μm) has a higher surface/volume ratio than the rods of lower vertebrates. If these factors favour

relatively more rapid lowering of $[Ca]$, by Na/Ca exchange, a single photon can, in effect, completely light-adapt the small compartment where the inward current is suppressed. This idea is consistent with results from rabbit retina showing that flashes even at threshold intensity cause acceleration of rod guanylate cyclase¹⁴.

The intracellular messenger hypothesis of phototransduction has come almost full circle since first calcium⁷ and then a cyclic nucleotide¹⁵ were proposed for the role. Both hypotheses have generated enormously productive research programmes, each acting as a foil for the other. The story now seems to be coming to a close with cGMP the excitatory messenger and Ca^{2+} a feedback messenger for recovery and adaptation. But without the work stimulated by the original calcium hypothesis, the present satisfying resolution of the mechanism of phototransduction might have been much longer in coming. $\square$

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Edward Pugh is in the Department of Psychology, University of Pennsylvania, 3815 Walnut Street, Philadelphia, PA 19104-6196, USA, and Jennifer Altman is an assistant editor of Nature.

Materials science

A final limit to superheating

Robert W. Cahn

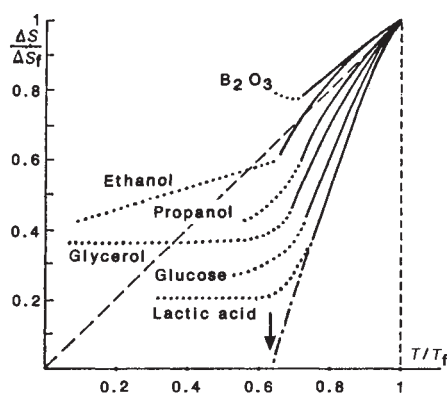
METASTABILITY is the crucial feature of all phase transformations. At the exact thermodynamic equilibrium temperature between two phases, the rate of transformation is nil, and only by superheating or supercooling into the range of metastability can the transformation be driven at a finite rate. Even so, kinetic hindrance arising from slow atomic diffusion can brake or entirely prevent a transformation, even when the thermodynamic driving force is substantial. The amount of superheating or supercooling with respect to the thermodynamic transformation temperature which is feasible in practice is therefore defined by kinetic considerations. On page 50 of this issue¹, Fecht and Johnson ask whether kinetics are the only relevant consideration, or whether there is such a

thing as an absolute metastability limit, a temperature above (or below) which a metastable phase is forced to transform. They find that entropy gives an upper limit to the superheating possible for crystalline materials.

Fecht and Johnson¹ start with a long-established curiosity in the theory of glasses, the Kauzmann paradox². Kauzmann pointed out that if the entropy of a liquid, plotted as a function of temperature, is extrapolated into the temperature range in which the liquid congeals as a glass (where configurational entropy is in fact frozen) then generally the extrapolation shows a temperature, well above absolute zero, at which the entropy of the extrapolated liquid equals that of the crystal (see figure).

Kauzmann went on to advance reasons why, if a glass could maintain its configurational equilibrium down to the critical or 'Kauzmann' temperature at which its entropy equals that of the crystal, it would be forced to crystallize instantly. This is a paradox because at such low temperatures atomic diffusion is extremely sluggish so that crystallization should be impossible. Of course, as Kauzmann pointed out, the paradox is actually a metaphysical one, because it would be kinetically impossible for a glass to maintain configurational equilibrium down to such a low temperature. But the Kauzmann paradox continues to fascinate glass theorists and they continue to discuss its implications^{3,4}.

It has long been recognized that, whereas a liquid can be metastably super-



Variation of entropy (normalized with respect to the entropy of fusion) with temperature (normalized with respect to the freezing point). Solid curves, liquids in equilibrium; dotted curves, glassy state. Arrow, isentropic temperature for lactic acid. (Based on Kauzmann's data, from ref. 8.)

cooled, sometimes through hundreds of degrees, so long as heterogeneous nucleation can be discouraged (for example, by dividing up the melt into tiny discrete droplets), normally a crystal cannot be metastably superheated by more than a fraction of a degree. It can be superheated briefly, of course, while latent heat flows into the solid, but such a kinetic transient has nothing in common with metastable superheating. More recently, it has been recognized that melting, like freezing, is usually nucleated heterogeneously, at surfaces or interfaces (see my News and Views article⁵), and when special tricks are employed to prevent this then substantial superheating is feasible, for instance with noble-gas crystallites embedded epitactically in a metal crystal.

Fecht and Johnson discuss in this issue¹ whether there is an intrinsic limit to such superheating, just as the Kauzmann paradox sets an intrinsic — albeit metaphysical — limit to the supercooling of a glass. They follow Kauzmann in seeking to locate an isentropic temperature, T_i^s , at which the entropies of the superheated crystal and the liquid phase are equal.

Fecht and Johnson calculate the entropy of superheated crystalline aluminium. It turns out that the role of lattice vacancies is crucial. Their concentration increases with rising temperature; if their contribution to entropy is neglected, then T_i^s coincides with the melting point, T_m , and superheating would be excluded. Taking the configurational entropy of vacancies into account yields an isentropic temperature (also the crystalline instability temperature) of $1,292\text{ K} = 1.38T_m$. (Fecht and Johnson's Fig. 1 on page 50 of this issue shows the result of the calculation; both upper and lower isentropic temperatures appear, the latter being the one associated with the Kauzmann paradox.)

If the vibrational entropy associated with vacancies is also allowed for, T_i^s comes out a little lower, at $1,234\text{ K}$. An isenthalpic temperature ($1,217\text{ K}$) can also be calculated; above this temperature, melting is no longer heat-flow limited. Indeed, it is hard to see what can prevent a crystal from spontaneously melting in the narrow temperature range between the isenthalpic and the isentropic temperatures.

The vacancy concentration at the instability temperature is found to be as high as 6–7 per cent. This calls to mind earlier experimental and theoretical results (mostly due to Górecki; see my News and Views article⁶) suggesting that melting is correlated with the achievement of a critical vacancy concentration (taken to be less than 1 per cent by Górecki). But the effective vacancy (hole) concentration in an equilibrium melt at the melting point is estimated at around 10 per cent, and one way of regarding the isentropic catastrophe at $1.38T_m$ is that the solid becomes unstable against spontaneous collapse because of the high vacancy concentration. Fecht and Johnson express this by pointing out that at this temperature, the specific volumes of the liquid and of the superheated crystal become identical.

The authors conclude their stimulating *jeu d'esprit* by extending their instability concept from a pure metal to a solid solution, and present a hypothetical entropy diagram which shows T_i^s as a function of solute concentration.

The notion of what is in effect an upper absolute instability limit for the existence of a crystalline structure is a novel one: perhaps the closest parallel is the concept of an upper absolute instability limit for a first-order order–disorder transition⁷. □

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Robert W. Cahn is in the Department of Materials Science and Metallurgy, Cambridge University, Pembroke Street, Cambridge CB2 3QZ, UK.

Daedalus

Tricks of the trade

BECAUSE the real world has few economically significant cyclic variations, business cycles must ultimately derive from human psychology. The big global cycles are probably too remote from individual business decisions to be easily explained; but Daedalus has hopes of understanding the small-scale fluctuations of small groups of businesses. DREADCO's economists are studying a specific case — a group of companies all competing to sell particular products in the same market: one of DREADCO's markets, in fact. The team is Fourier-transforming past records of sales, prices and so on, into their equivalent 'economic spectra'.

Each frequency peak in a firm's spectrum will correspond to some characteristic mechanism within it. Thus a two-year cycle may indicate how long a manager takes to disown his previous opinions; a one-year component may reveal the period over which a new technical advance comes to seem routine. Peaks shared by two companies show the influence of one firm on another: in fact the DREADCO team's final model of the system resembles a net of coupled oscillators.

The point of this laborious exercise is to initiate a new and subtle form of economic warfare. Somewhere in the spectrum of each of his rivals, says Daedalus, there should be a strong peak corresponding to some quite unstable attribute — some optimism or pessimism, some expansionist or defensive outlook poorly coupled to material reality, through which that rival is vulnerable. The economic model should soon reveal what behaviour on DREADCO's part (a periodic shift in pricing structure at the crucial frequency, maybe) will drive the rival concern into hopeless and uncontrollable resonant oscillation, until it goes bankrupt at one of the extremes of swing.

But what will happen when Daedalus's rivals realize the trick and try to play it on him? His defence is simple: bureaucracy. This damps and delays all organizational change. Too little, and wild instabilities are possible; too much, and the system cannot respond adequately to change. So Daedalus is sabotaging DREADCO's computer net, inserting formal paperwork and bumbling committees at strategic points, making the firm critically damped in all modes of oscillation. It will then have the minimum response-time consistent with stability.

Thus DREADCO's whizz-kid rivals will bite the dust, victims of their own heedless modernism: while DREADCO itself, like many other apparently old-fashioned organizations, will continue on its stately path into the future.

David Jones

Species selection: its range and power

SIR—Loquacious and contentious primates that we are, too much of human debate concerns words rather than things. Yet terms and concepts must be clarified if we hope to gain any proper understanding of phenomena. Species selection is an important and confusing concept lying at the heart of attempts to reformulate evolutionary theory as a hierarchy of interacting levels^{1,2}, rather than (as darwinian convention holds) a nearly exclusive proposition involving struggle among organisms for differential reproductive success.

Maynard Smith³ accused us of overextending the potential role of species selection by proposing it as a source for the origin of complex morphological adaptations. We agreed⁴ that species selection could not work in such a manner, and pointed out that all proponents of the idea had always so acknowledged. (This conclusion is a simple statement of probability. Complex morphological adaptations, requiring the sequential accumulation and integration of hundreds of parts, cannot arise as the fortuitous side-consequence of a causal process acting at some other level — gene or species, for example. Such complexity must be causally built by selection working directly on the phenotypes of organisms.) Maynard Smith⁵ responded by citing quotations supposedly indicating our previous belief in species selection as a cause of complex morphological adaptations, and welcomed us back to sensible orthodoxy.

Changing one's mind is among the world's most salutary blessings, and a badge of valour and virtue — so we would be happy to acknowledge Maynard Smith's interpretation, if it were true. But we have not changed our position on species selection; we, and all palaeontologists involved in formulating this concept, have always recognized that it cannot explain complex morphological adaptations.

Maynard Smith's quotations simply illustrate a misunderstanding in the use of terms. The quotations all advocate species selection as a cause of palaeontological 'trends' and Maynard Smith has equated trends with complex adaptations. Not so. In our original paper on punctuated equilibrium⁶ we defined trends as "biostratigraphic character gradients" — the standard palaeontological usage. Most empirical trends in fossils are chronological gradients in simple characters, the most famous examples being trends towards increased body size expressed as Cope's rule⁹. Indeed, it is the chief frustration of the fossil record that we do not have empirical evidence for sustained trends in the evolution of most complex morphological adaptations — the jaws and eyes of vertebrates, to cite two classic cases. Thus, palaeontological trends,

properly defined, are the very aspects of morphology that are most subject to potential explanation by species selection, because trends are simple, sustained changes that can arise by hitchhiking on a process of sorting among species.

The acknowledgement that species selection cannot produce complex morphological adaptations is no vitiation of its power or importance in evolution, but only a mark of avoiding a 'category error' in understanding its action. Forces are appropriate to their levels; gravity is not unimportant in the Universe as a whole because quarks hardly notice it. Similarly, much of the genome, including 'selfish' DNA and 'outlaw' chromosomes, probably arises by gene-level selection, whereas the great patterns of differential diversity and its geological fluctuations are strongly impacted by species selection (can we really hope to explain why the world holds more than a million species of insects and only a dozen or so of priapulids by relative adaptive success of their equally complex morphologies alone?). But hierarchy theory is also fascinating because levels interact, and phenomena generated at one level have important effects at others. Thus, duplicated genes may be a prerequisite for flexibility in the evolution of morphological complexity, but they probably arise by genomic selection. And simple, sustained chronological gradients in morphology — the classical 'trends' of palaeontology — may be produced as effects of downward causation from species selection.

Maynard Smith⁷ ends by welcoming us back to his conceptual edifice. But while he was out crusading for his castle, the building was growing. The darwinian ground floor is as vibrant as ever, but a wonderful basement has been added for gene and cell-lineage level selection¹⁰ — and a lovely attic for the level of species. The view from the top is puzzling, but endlessly fascinating.

STEPHEN JAY GOULD

*Museum of Comparative Zoology,
Harvard University,
Cambridge, Massachusetts 02138, USA*

NILES ELDRIDGE

*American Museum of Natural History,
New York, New York 10024, USA*

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Examples, please

SIR—The most appropriate usage of the word 'homology' has been a continuing topic in the literature. One argument against restricting it to its precise, historical, biological meaning of common ancestry is that, in molecular biology, if it's similar it's homologous because you can't get molecular similarity on the basis of convergent evolution. I believe the persuasiveness of that argument depends largely on the lack of awareness of the (possible) occurrence of analogy at the molecular sequence level, and would like to collect examples. Accordingly, I would be grateful if readers could send me any instances they know of in which similarity is not the result of common ancestry.

WALTER M. FITCH

*Department of Biological Sciences,
University of Southern California,
Los Angeles,
California 90089-1340, USA*

Neither waves, nor particles, but quantons

SIR—In discussing¹ the continuing debate on the foundations of quantum theory and, more specifically, the physical nature of quantum entities, John Maddox summarizes the question by opposing "Bohr's opinion, now called the Copenhagen view, . . . that such an entity is . . . either a wave, or a particle" with "the contrary view, due to De Broglie, . . . that a quantum entity is both a wave and a particle". One might argue that Bohr's ideas were more subtle (and rather more obscure), but the description no doubt fits the widespread quantum vulgate, contrasting the two views — and only these two.

It is not surprising that the brave explorers who first landed on the quantum continent, more than 60 years ago, stumbling on its rather weird inhabitants, tried to describe them by reference to more familiar company. To classical or post-classical physicists there were only two types of physical entities, namely particles and waves. Since the strange quantum beings showed a behaviour reminiscent sometimes of particles and sometimes of waves, it was not unnatural to discuss them in classical terminology, under the philosophical cover of the *ad hoc* 'wave-particle duality'.

The conquistadors, discovering in the Andes a woolly and lipped animal looking somewhat like a sheep and somewhat like a camel, did not for long resort to camel-sheep duality and soon used a native word, lama, to describe it. Nor did the gold-diggers of Australia keep to a duck-rabbit duality for describing the furry duck-billed platypus. Despite partial similarities, it was soon clear that these were

beasts in their own way.

We are now familiar enough with the quantum entities. We know that wave and particle descriptions give us but partial views. For a wave is continuous and extended, a particle is discrete and localized, while quantum beings are discrete and extended. It is only in very special circumstances, for a rather restricted set of experimental devices, that the wave-particle duality suffices to give us a satisfying account. To understand most modern experiments, we must use the full-fledged apparatus of quantum theory to which the wave-particle duality has no relevance whatsoever. Even rather simple experimental situations do not lend themselves to natural explanations in terms of wave and particles, whether in alternative (either . . . or . . .) or comprehensive (both . . . and . . .) use^{2,3}.

It seems more than time to recognize that quantum entities are *neither* waves, *nor* particles. As the basic objects of a non-classical theory, they certainly deserve a new, non-classical, and specific name⁴. A natural solution is to encompass all their species, photon, electrons, baryons, gluons etc., under the common appellation of 'quantons', as long advocated by Bunge and others. This would not only dispose of the cumbersome and ill-defined 'wave-particle duality' but would also offer definite pedagogical help by stressing for the student the radical novelty of quantum theory and the danger of naive classical pictures⁵.

Finally, it would remind us that the debate about the foundations of quantum theory is not only a matter of interpretation and that there remain open questions concerning the physical theory itself⁶. The main problem is to understand how large pieces of matter built out of quantons can behave like waves, or like particles or like neither. In other words, rather than interpreting quantum theory, we need to understand classical theories, that is, to master the conditions of validity of classical theories⁷. It is to be expected that such an understanding will pave the way to a complete solution of the most irritating riddle of quantum theory, that of the 'reduction of the state vector' under a measurement, which could be but an efficient recipe following from the macroscopic nature of the measuring apparatus.

J.-M. LÉVY-LEBLOND

*Physique Théorique,
Université de Nice,
Parc Valrose,
06034-Nice Cedex, France*

Cystic fibrosis allele segregation

SIR—Kitzis *et al.*¹ presented data on normal siblings of cystic fibrosis (CF) patients, suggesting segregation distortion of the CF allele with sex. In their study, 20 of 22 normal homozygotes were girls, and 16 of 21 heterozygotes carrying the paternal CF chromosome were boys. In contrast, the maternal CF chromosome had been passed equally to boys and girls. The authors suggested that there is preferential inheritance of the CF allele from male to male, but this conflicts with the generally observed equal proportions of male and female CF patients. Further data were requested to determine whether their result can be confirmed.

In a sample of comparable size consisting of normal siblings of Dutch CF patients, we find no indication of an abnormal segregation of the CF allele: 10 of the 20 normal homozygotes are girls and 11 of 19 heterozygotes with the paternal CF chromosome and 13 of 18 heterozygotes with the maternal CF chromosome are males.

The study of Kitzis *et al.* was motivated by the repeatedly reported suggestion of increased fertility of CF carriers. About an equal number of studies, however, do not confirm this suggestion^{2,4}. These studies are seldom cited. It has also been shown on several occasions that sampling of families through affected children introduces a bias in favour of larger families^{5,6}. Methods for correction of this ascertainment bias are available^{5,6}.

L. P. TEN KATE
G. J. TE MEERMAN
C.H.C.M. BUYS

*Department of Human Genetics,
University of Groningen,
Groningen, The Netherlands*

D.J.J. HALLEY
B. OOSTRA

*Department of Clinical Genetics,
Erasmus University,
Rotterdam, The Netherlands*

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SIR—Kitzis *et al.* (*Nature* **333**, 215; 1988) presented interesting and unusual data on the segregation of the cystic fibrosis (CF) mutant allele to male and female progeny. However, the explanation and implications proffered are not consistent with their results. They found that, of the phenotypically normal progeny of (presumably) heterozygous parents, most homozygous normal progeny were females, most heterozygotes carrying the

paternal CF allele were male, and that male and female heterozygotes carrying the maternal CF allele were equally represented. The authors claim that, because there is preferential inheritance from male to male, that this provides a mechanism for expansion of the CF allele in caucasian populations which exhibit unexpectedly high frequencies of this otherwise deleterious allele. Closer examination of their data shows that the ratio of mutant to normal alleles in the progeny (38:82) is close to the expected 1:2. There were 21 heterozygous progeny carrying the paternal CF allele compared to the 20 expected. There is no mechanism here which would promote CF mutants over normal alleles.

The authors go on to suggest that the CF allele may be linked to some factor causing differential sperm viability or embryo survival. If so, one would expect the overall frequency, but not the sex ratio, of heterozygous offspring carrying the paternal CF allele to be affected. To explain the distorted sex ratio of these heterozygotes, the factor affecting viability would have to have differential effects depending on the presence of the paternal X or Y chromosome. The difficulty would be in explaining why there is such a marked deficit of male homozygous normal progeny.

An associated segregation of the CF allele (on chromosome 7) and the Y chromosome would explain the observed imbalanced sex ratios. However, one would also then expect more females than males among heterozygous offspring carrying the maternal CF allele. The authors do not give results for this class of offspring, other than to say that equal numbers of males and females were observed (which is strictly impossible as there were 17 progeny in total). Assuming that the number of females differ from the number of males by only one, either way, fitting the observed row and column totals and the observed average association between the CF allele and the Y chromosome, gives a residual $\chi^2 > 7.63$, $P < 0.01$, thus failing adequately to explain the observations. This explanation would also require that most CF homozygotes were male, which is known not to be the case.

The conclusion must be that the results of Kitzis *et al.* are puzzling and not explained by any simple mechanism. Nor do they explain the surprisingly high frequency of the CF allele. Further speculation on the matter should probably be left until these results are confirmed, or otherwise.

JOHN P. GIBSON

*Centre for Genetic Improvement of
Livestock,
Department of Animal and Poultry
Science,
University of Guelph,
Ontario, Canada N1G 2W1*

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The obvious difference

Keith Percy

Intellectual Property Rights in Biotechnology Worldwide. By Stephen A. Bent, Richard L. Schwaab, David G. Conlin and Donald D. Jeffery. *Macmillan, London/Stockton, New York: 1987. Pp. 640. £65, \$130.*

THE PATENT authorities are at last beginning to address the problems peculiar to biotechnological inventions. The US Patent Office has led the way in granting patents on animals and plants, and giving monopolies of often generous scope on products of recombinant DNA and monoclonal antibody technology. By contrast, at the European Patent Office and in Britain, decisions adverse to the patent proprietor have been handed down in the case of two recombinant DNA inventions, only narrow claims to hybridomas have been allowed, the first European patents for genetically engineered plants have only very recently been allowed and there has yet to be any pronouncement on the patentability of animals.

In *Intellectual Property Rights in Biotechnology Worldwide*, four US patent lawyers have produced a book which will be of great value to patent advisers and helpful to those concerned with plant breeders' rights. Frankly, it will be too stodgily legalistic to be read right through by most scientists, but this is no criticism: biologists need a different kind of book altogether.

Viewed globally, the patenting of a recombinant DNA product having the activity of a known protein is in a mess. Many patents have been issued in the United States, largely because the objection of 'obvious to try' does not apply in US law. Thus, patent applicants in the United States have succeeded by showing the examiner that the recombinant method of preparing a cDNA posed problems, for example in the low abundance of a particular mRNA or in the difficulty of using pools of oligomers, containing all the nucleotide sequences encoding a known amino acid sequence, as hybridization probes. The argument then runs that it could not be predicted that the desired product could be made and therefore that the product itself is 'unobvious'. The authors give the history of some cases in the United States to illustrate these successes. They do not, unfortunately, balance the discussion by explaining the very different European situation, or by looking to the future when these arguments will be redundant because improved techniques will have removed many of the snags in gene cloning.

Current European practice must be baffling to the scientist. Large numbers of applications for the cloning and expres-

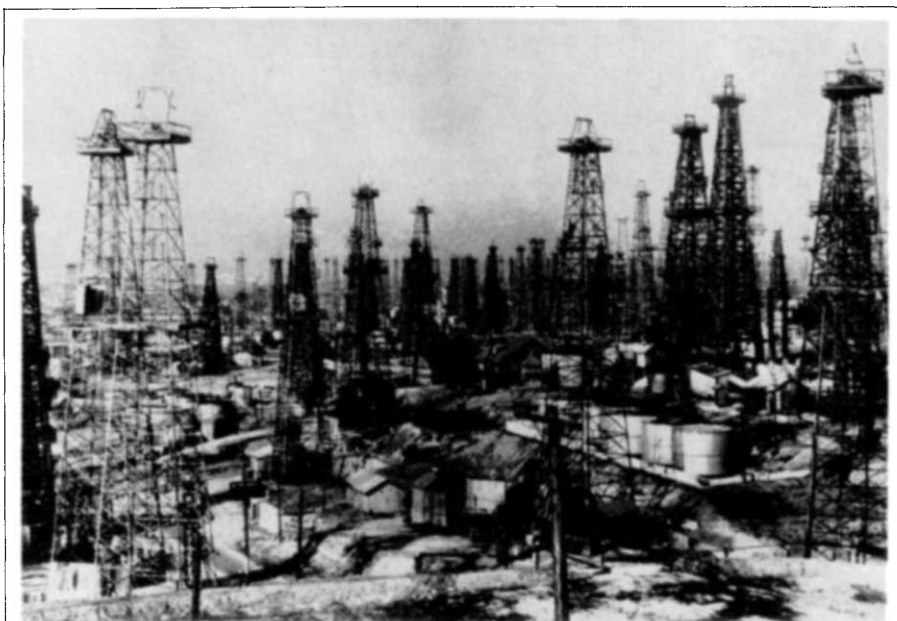
sion of genes coding for known proteins are being filed, yet few are emerging as patents. Part of the reason is that there is a backlog created by a shortage of examiners, but the principal factor is the examiners' objection that the recombinant protein and the cDNA encoding it are inherently 'obvious'. Everyone knows the protein to be desirable and therefore attempting to make it by standard recombinant methods is obvious. This was the core of The Wellcome Foundation's case, in its application to the UK Patents Court to annul Genentech's UK patent on tissue plasminogen activator (TPA).

Although the Wellcome decision was issued as recently as July 1987 (the same month in which the book went to press), Bent and his colleagues have managed to add a brief commentary on the case. Not only were Genentech's claims to "recombinant human TPA essentially free of other proteins of human origin" and "human TPA unaccompanied by associated native glycosylation" found invalid because they were considered to be excessively broad, but a third claim to "human TPA as produced by recombinant

DNA technology" was rejected both as being too wide and as being a claim to "an obviously desirable and potentially possible end". There are points to criticize in the decision, which is under appeal, but I believe that the result was correct because it was indeed obvious to attempt the preparation in the expectation that any difficulties such as the low abundance of the mRNA could be overcome.

Assuming that the court's view is upheld on appeal, the future for patenting this kind of invention looks bleak. A patent has to provide a 'consideration' for the monopoly; in other words, it has to benefit the public through the teaching of the specification. The width of monopoly granted has to be commensurate with that teaching. Traditionally, the 'consideration' has been the details of how to prepare and use a chemical compound, construct a piece of machinery and so on. Yet, where a known protein is being made by a standard recombinant method, the benefit is merely intellectual information — that is, the nucleotide sequence of the DNA. This information is purely of a mental character, analogous in principle to spectroscopic data for a chemical compound, rather than constituting the instructions on how to make the product. The patent system is not currently designed to give rewards for mental information and it has been suggested that the law should therefore be changed.

As an alternative to applying for a patent, the originator of an invention can keep the invention secret. If some form of



View from the hill — Signal Hill oilfield in southern California, around 1935, crowded with the new rotary drills. This innovation enabled the drilling of holes much faster and deeper than previously, and for the first time provided geologists with continuous samples. The picture is taken from *Sand* by Raymond Siever, which shows how the properties of sand — the shape of its grains, its chemical-mineral composition and its radioactive age, and its location in the Earth's crust — are powerful clues in understanding the dynamics of the Earth's surface. The book is in the *Scientific American Library* series, and is distributed by W.H. Freeman, price \$32, £14.95.

broad patent right is not available for recombinant DNA products, this will become an increasingly attractive option for companies. Academic scientists, who naturally wish to publish their work, including DNA sequences, would find this idea detestable. But hybridomas and also microorganisms used to produce traditional compounds are already often kept under wraps. It is therefore good to find that the authors have included two chapters on trade secrets and physical property rights, the second summarizing the relevant civil and criminal law in 15 countries.

Another strength of the book is its excellent treatment of plants. The US Patent Office changed its policy in 1985, when it allowed a patent for maize plants having increased levels of tryptophan, a stably inherited characteristic produced from mutant cells. In so doing it over-rode the principle that if plant variety protection could be obtained, patent protection could not be obtained. The European equivalent of the problem is about to be resolved. The patent law was drafted with the intention of excluding plant varieties from patent protection, a result of a long-standing tussle between the respective authorities. The genetic alteration of plants by non-horticultural methods introduces the same new traits into many varieties. Is patent protection to be denied because a claim to a large family of plants having such a novel trait inevitably includes 'varieties'? The authors say not, in the typical campaigning style which

makes their work such a pleasure to read, and the European Patent Office is about to prove them right.

In grappling with the issue of what kinds of use would infringe a patent to a plant or animal, there are timely comments on whether a farmer could save the seed from the genetically altered plants or breed from the novel animal. The recent grant of a US patent for a transgenic mouse heralds a whole range of new questions. One of my colleagues handling such an invention gave his client some advice from right outside the textbooks — install an electric fence.

The first two chapters of the book contain little but conceptual waffle, and the index is very poor; I keep trying to find the scattered references to the "reverse doctrine of equivalents" — how to interpret a seemingly broad claim more realistically, the one word "Interferon" being a splendid example. The country by country summaries of patentable subject matter, microorganism deposit requirements and plant variety protection are invaluable, however, and altogether the authors have gone a long way to creating an authoritative work of reference for patent agents dealing with biotechnology. For their part, biologists would more likely benefit from R. S. Crespi's *A Basic Guide to Patenting in Biotechnology*, due from Cambridge University Press towards the end of the year. □

Keith Percy is a Chartered Patent Agent with the British Technology Group, 101 Newington Causeway, London SE1 6BU, UK.

High-level affairs

Patricia Smith Churchland

Computer Models of Mind: Computational Approaches in Theoretical Psychology. By Margaret A. Boden. *Cambridge University Press: 1988. Pp.289. Hbk £30, \$42.50; pbk £10.95, \$15.95.*

THE BRAIN is something of an embarrassment to theoretical psychologists — a bit like the rich uncle who foots the bills, but whose rough manners and roguish ways are not quite what is wanted in polite company. By and large, psychologists are, of course, not dualists. They know as well as anyone that it is the brain that deals with perception, reasoning and learning; that, to put it succinctly, mental processes are brain processes. Yet theoretical psychology is characterized, rather pointedly, as the science of the mental, in contrast to neuroscience, the science of the brain.

Given the absence of a non-physical psyche, this characterization of the division of labour might seem a throwback to the bad old cartesian heyday. But con-

temporary psychologists justify the division of labour by invoking the idea that there are different levels of organization in nervous systems, and that properties of higher-level organization are distinct from, although produced by, the events and processes on lower levels. Mental processes are presumably high-level affairs, and theoretical psychology aims to discover the principles that operate at this level. Although mental processes are indeed processes of the brain, high-level principles are different from those governing dynamics at the cellular or the circuit level. Thus — and an aerodynamic prosthesis should be strapped on for this leap — the brain can conveniently be left out of consideration in generating theories of cognitive functions. The important constraints on theories are derived from behavioural studies of capacities such as language processing and reasoning, and from computational considerations.

Computer models that purport to simulate high-level processes now dominate the theoretical niche in psychology. In *Computer Models of Mind*, Margaret Boden provides an eloquent discussion of their general rationale, their philosophical assumptions and their history. Apart from

the introductory and concluding chapters, which give her own view of things, the main body of the book is a kind of bestiary of some of the models that have been invented to explain selected features of cognition — pattern recognition in vision, language processing, problem solving and learning. Wondrous computational beasts, displaying mixtures of cunning solutions and crashing defects, are trotted out and briefly described; Boden introduces each of them in the context of the problems the model is meant to address, and she provides useful commentary on their strengths and weaknesses and how they compare to one another.

The survey is broad and well researched, though occasionally the explanation of the models and the accompanying critique are a bit too condensed to be followed by the novice. Additional illustrations would have been helpful in this respect, but the ample references permit further exploration on one's own.

How successful are these computer models? Not very, as Boden carefully points out. As yet there is no model that comes even close to simulating a human cognitive capacity. So, in a sense, the bestiary is a catalogue of 'never-wuzzers'. Nonetheless, a great deal has been learned as various hunches have been played out, and ideas have been given their chance and laid to rest. More importantly, the computational complexity of certain processes, such as visual perception, are vastly better appreciated in light of our understanding what will *not* work. Building on the bones of the failures, Boden stresses, we improve the analysis of the task performed, and computational ideas correspondingly evolve. In any case, the enterprise has to start somewhere.

The question arises, however, as to whether or not that enterprise was guaranteed to fail from the start. Much of the work discussed in the book assumed that, at the cognitive level of organization, the appropriate architecture is that of a von Neumann machine. As Boden explains, this means that the processes are serial inasmuch as operations are performed sequentially, one at a time, and that the computations are manipulations of symbols by application of formal rules. Taken literally (and many researchers do exactly that) the assumption means that problem solving, visual perception and learning really amount to running a program on a von Neumann computer.

In the face of a long run of disappointing results within this framework, criticism of the assumptions fomented in the trenches and gathered force. Following the inspiration of early work by Warren McCulloch and Frank Rosenblatt, some researchers in computer science and computational psychology revived the idea that maybe the brain does matter after all. Perhaps, it was heretically conjectured, processing

even at high levels is done in parallel, and perhaps algorithms suited to a brain-like architecture would be more successful.

Network modelling (also known as 'connectionism', and 'parallel distributed processing') thus emerged from its 15-year eclipse, and now is probably at least as fashionable as conventional strategies. Boden gives a good introduction to contemporary connectionist modelling, but because the research is developing at a breakneck pace, her discussion is already rather dated. On the basis of extant models, she suggests that although connectionism appears to be more promising for some domains such as perception, it may have limitations in others such as language processing. She gives a balanced account of the debate between von Neumann die-hards such as Zenon Pylyshyn, who dismisses network models as irrelevant to cognition, and pioneering connectionists such as Geoffrey Hinton, who has laid down much of the conceptual and technical foundation for the new approach.

Does network modelling mean that neuroscientific considerations figure more prominently in theories of cognitive processing? Certainly they do so more than in conventional models. But connectionist models addressing cognitive problems typically honour only very general neurobiological constraints, and details of brain organization, neuron types, response profiles and so forth are entirely ignored.

Apart from the emphasis on cognition, however, there is a definite trend towards devising neurobiologically realistic models of complex, if lower-level, cortical tasks such as computing the shape of an object from its grey-level shading profile (see, for instance, Lehky and Sejnowski *Nature* 333, 452–454; 1988). Moreover, insights from connectionist models on such matters as population coding, distributed representations, fast and slow weights, and the relation between a neuron's receptive and projective fields are being used in understanding the computational properties of the nervous system.

Computational neuroscience is becoming a force to be reckoned with. It is (reasonably) beyond the scope of Boden's book, but it does promise the possibility of connecting links between computational psychology and neuroscience. More particularly, it may be a fruitful source of constraints for computational psychologists that will help to direct their search for general principles — because computational space is vast, such constraints should be viewed as a godsend. Like Boden, I think computational psychology has an exciting future, but I see it as a future more closely tied than ever to neuroscience. □

Patricia Smith Churchland is a Professor in the Department of Philosophy, University of California, San Diego, California 92093, USA.

In the buoyancy business

D. T. Donovan

The Natural History of Nautilus. By Peter D. Ward. *Unwin Hyman*: 1987. Pp.267. £35, \$34.95.

Nautilus: The Biology and Paleobiology of a Living Fossil. Edited by W. Bruce Saunders and Neil H. Landman. *Plenum*: 1988. Pp.632. \$95 (North America), \$114 (elsewhere).

Nautilus is the only living cephalopod mollusc which inhabits an external, chambered shell providing buoyancy, and it is therefore central for understanding the countless shells of similar type found in the fossil record. Its anatomy was first described, on the basis of a single specimen from the New Hebrides, by Richard Owen in 1832. Long regarded as rare and elusive, later attempts to investigate its mode of life, physiology and reproduction brought only partial success.

Modern work started in the 1960s with Anna Bidder's observations and X-rays of living animals in New Caledonia, and Eric Denton and John Gilpin-Brown's elucidation of the workings of the buoyancy mechanism. Since then *Nautilus* research has become a growth industry. Peter Ward, himself one of the leading investigators, cites about 80 papers on the subject published between 1976 and 1986.

In his book, Ward deals at length with shell structure, buoyancy, growth, distribution and ecology, matters with which he has been especially involved in recent years. He also gives a general description of the soft parts, but his account of the internal organs is brief, with no mention at all of the nervous system and sense organs. Saunders and Landman's edited work contains 37 chapters, Ward being one of the 48 authors involved. As well as the ground covered in Ward's book, this volume includes an important section on anatomy, physiology and metabolism, a more detailed account of reproduction and embryology, and a comprehensive history of research.

Has the *Nautilus* industry helped us to a better understanding of the extinct shelled cephalopods? *Nautilus* is indeed the most primitive of living cephalopods, both in possessing an external shell, and in its

nervous system and sense organs. Nevertheless, it is probably far removed from the earliest known cephalopods of the late Cambrian, which lived over 500 million years ago, and in the context of cephalopod evolution as a whole it must be seen as displaying a mixture of primitive and specialized characters. One of the specializations is the jet propulsion system, in which water is expelled from the mantle cavity by a combination of body retraction and funnel contraction, whereas in many Palaeozoic forms propulsion was probably by body retraction alone. In this respect it is disappointing that although both volumes address the evolutionary origin of *Nautilus* in taxonomic terms, neither does so in the light of functional morphology.

As regards the buoyancy system, there is a great deal in common between *Nautilus* and the other two living genera with (internal) chambered shells, *Spirula* and *Sepia*. Here *Nautilus* can help us in understanding extinct forms, and a good deal of Ward's last chapter, entitled "Paleontological Implications", is devoted to this aspect. He begins this chapter by outlining the phylogeny of the shell-bearing cephalopods, and includes two diagrams (Figs 7.2 and 7.5) which convey the same information but differ in details and name endings — surely confusing for the non-specialist. The ammonoids, which account for a substantial part of the concluding discussion, are not shown in any detail in either diagram.

Ward also considers the question of depth limitation for animals with partly gas-filled shells. The maximum habitable depth depends on the resistance of the shell and siphuncle to hydrostatic pressure, factors which have been tested in *Nautilus* and the results applied to extinct forms. Any cephalopod chambered shell is a compromise between strength and speed of liquid interchange (and hence change of buoyancy) — what Ward calls the "strength versus emptying paradigms". Many extinct genera are thought to have been restricted to depths shallower than the 700 m or so which *Nautilus* can tolerate. Recent work on the behaviour of liquid in the chambers, and on the function of the liquid-retaining pellicle which coats the septa, is beginning to throw light on buoyancy control in ammonites (where a similar pellicle was present) and perhaps on the significance of the complex ammonoid septa, which may have had a liquid-retaining function as well as a mechanical one.

An interesting aspect of *Nautilus* is its longevity. Different species may take from five to eleven years to grow to maturity, and it has been suggested that some of them may live up to 20 years of age. A growth-limiting factor is the time needed for each successive septum to become thick enough to withstand external

• *Perceptrons* by Marvin L. Minsky and Seymour A. Papert has just been re-issued by MIT press in an expanded paperback edition, dedicated to the memory of Frank Rosenblatt. *Perceptrons* was the first systematic study of parallelism in computation and has remained a classical work; the new edition contains an epilogue on the revival in connectionism, and a prologue which gives a view from 1988. Price is \$16.95, £11.25.

hydrostatic pressure and so allow growth to continue. It is uncertain how far this applies to extinct forms; many early Palaeozoic cephalopods had thin shells and septa, and the thick shell and septa of *Nautilus* may be specializations to permit survival at considerable depth, at the price of slower growth.

Ward's book contains a stimulating

summary of present knowledge which palaeontologists will find particularly useful. Zoologists who wish to have full information on anatomy and physiology will doubtless turn to Saunders and Landman. □

D.T. Donovan is in the Department of Geological Sciences, University College London, Gower Street, London WC1E 6BT, UK.

Reacting to change

I.W.M. Smith

Molecular Reaction Dynamics and Chemical Reactivity. By Raphael D. Levine and Richard B. Bernstein. *Oxford University Press: 1987. Pp.535. Hbk £25, \$39.95; pbk £15, \$18.95.*

CHEMICAL reaction dynamics is concerned with the intricacies of chemical reactions at the molecular level. The field was born in the early 1960s as chemists began to apply molecular-beam techniques and sensitive spectroscopic methods, and as the development of large, high-speed computers enabled them to simulate sufficient numbers of molecular collisions to give their calculations statistical significance.

These studies, and their successors, have revolutionized the way in which chemists visualize chemical changes. We are no longer constrained to think only about how fast chemical species transform at a particular temperature, but we can ask, and often understand, how reactions might be influenced in rate or path by particular forms of energy, how the energy actually released as chemical bonds reform appears in the reaction products, and how the answers to these questions and many others are related to the way in which the forces between the atoms change as they dance around one another during the course of the molecular rearrangement.

Raphael Levine and Richard Bernstein's *Molecular Reaction Dynamics*, published in 1974, celebrated the advent of this vigorous area of research and had a richly deserved success. It introduced many students to the field and was a source of guidance and enjoyment to many of its experienced practitioners. Since 1974, however, there have been spectacular advances. Perhaps the most notable of them have resulted from the applications of lasers, especially the tunable dye laser; using techniques such as laser-induced fluorescence and resonance-enhanced multiphoton ionization, it is now possible to prepare and detect species in defined internal states with great sensitivity. The long-heralded marriage of spectroscopic and molecular-beam techniques has not only been consummated but has also

become a more equal partnership with the passage of time. There have, too, been parallel advances in beam technology. Developments in the design of nozzle sources have led to beams of greater intensity, defined and definable velocity, and cold internal temperatures, as well as the generation of weakly bound dimers and clusters.

More, surely, is to come, yet the reaction dynamics research community, especially its new recruits, will be grateful to Professors Levine and Bernstein for choosing this moment to revise their earlier text. The style, format and typeface are familiar and give some feeling of *déjà vu*, but this is misleading. This really is a new book, not just a revision, providing a panoramic and impressively up-to-date view of the subject. It is all done with great enthusiasm and panache, fulfilling the authors' aim of introducing "the language and spirit of the subject".

The organization of the book leads the reader through systems of increasing molecular complexity. After an introductory chapter, explaining what molecular dynamics is about, the concept of molecular collisions and how the dynamics in these collisions reflect the underlying intermolecular forces or potential energy surface are given a central role and are considered in some detail. One disadvantage of this approach is that molecular complexity and conceptual difficulty do not go hand in hand, and it is not until Chapter 4 that the link is established between the microscopic world of molecular collisions and the macroscopic sphere of reaction rates and rate constants. Newcomers to the subject may need some encouragement and guidance to help them connect the material in the early chapters with what is taught in most first-degree courses.

This is a minor quibble — altogether, the book is an impressive achievement. It brings the reader right to the forefront of the subject, the last chapter bristling with descriptions of new advances in multiphoton effects, molecular clusters, gas-surface scattering and stereospecific dynamics. Finally, it is a pleasure to record that this volume has been published at a price that students, and their elders, can afford. It will be widely read. □

I.W.M. Smith is a Professor in the Department of Chemistry, University of Birmingham, PO Box 363, Birmingham B15 2TT, UK.

Ups and downs in animal life

Tim Clutton-Brock

Social Behaviour in Fluctuating Populations. By Andrew Cockburn. *Croom Helm: 1988. Pp. 239. £30.*

MAMMALS can be divided into two categories: those that you can see but cannot catch, including most large species; and those that you can catch but cannot see, including most small ones. Because of the problems of visibility, research on the ecology of small mammals has concentrated largely on population demography based on samples of trapped animals. The dramatic population cycles of some species have provided an important stimulus to research in this area, and are at the hub of several controversies, but their causes remain elusive: cycles do not appear to be controlled in any simple fashion by food availability or predation, nor by changes in the frequency of aggressive genotypes in the population. As a result, it is commonly argued that cycles must be driven by behavioural responses to population density, involving reproductive suppression, interference or dispersal. The importance of understanding social organization and reproductive behaviour is consequently widely emphasized.

Cockburn's book provides an excellent review of the behaviour and ecology of cyclical mammals which should be of interest to a wide range of ecologists. Drawing on an extensive knowledge of experimental research on captive rodents, as well as field work on wild populations, he interprets existing knowledge of the life histories, ranging behaviour, mating systems and reproductive physiology of cyclical species within the framework of evolutionary theory. His treatment of the field's controversies is clear and balanced.

Cockburn's principal contribution to arguments over the causes of population cycles is a provocative but well-reasoned rejection of behavioural models. In the final chapter he considers whether a general theory of population cycles is possible, pinning his hopes on more complex models of the interaction between populations and their food supplies which incorporate intra- and inter-seasonal fluctuations in food availability. He may be right — but the great diversity of breeding systems and social organization which he documents so clearly carries the obvious implication that demographic processes should be expected to vary widely between species as well. □

Tim Clutton-Brock is in the Large Animal Research Group, Department of Zoology, University of Cambridge, 34A Storey's Way, Cambridge CB3 0DT, UK.

Faraday and the funding of science

In his Dibleby Lecture, which was shown on British television on 10 April, Sir George Porter argued for the value of 'pure' research and took Michael Faraday's innovative work on electromagnetic induction as an exemplary case. The British prime minister,

Mrs Margaret Thatcher, also holds Faraday dear. Here Geoffrey Cantor questions whether Mrs Thatcher has the right hero, while overleaf Tony Gardner-Medwin provides a sceptics' guide to the worth of curiosity-based research.

The scientist and his values

Geoffrey Cantor

IN HIS address, Sir George Porter chastised the British government for its myopic pursuit of affluence and its failure to recognize the crucial role of scientific research. He argued that scientific research—particularly pure research—is essential for the long-term success of a nation's technology, and thus of its economy, and that it must not be jeopardized by a preoccupation with goals set solely by short-term economic considerations.

Sir George began with several historical examples and quotations taken from leading British scientists of the early nineteenth century—David Brewster, Charles Babbage and, most prominently, Michael Faraday. Faraday, claimed Sir George, had been engaged in rather fruitless and time-consuming experiments in the late 1820s, at the behest of a committee of the Royal Society. These were aimed at improving optical glass and its means of manufacture. Exasperated by this work, Faraday relinquished it in July 1831 in order to pursue a line of pure research which had been on his mind for some time. A mere two months later this research reached fruition with his momentous discovery of electromagnetic induction. As we know, this discovery has been crucial for a wide range of technologies of great economic importance.

Sir George is not alone in invoking the name of Faraday. Several months ago the Prime Minister was interviewed on the television programme *Favourite Things*, and was asked to name her historical hero. Surprisingly, she did not choose a politician but a scientist. Nor did she choose Newton, despite his association with Grantham, her place of birth. Instead she named Faraday, and admitted that she had moved a bust of her hero into Number 10 Downing Street.

Yet Mrs Thatcher's Faraday is not the same Faraday offered to us by Sir George. She views Faraday as a genius who succeeded by virtue of his strength of character. Her Faraday is a Smilesian figure who transcended his humble background to

become the most successful scientist of his generation. She particularly stressed that he succeeded with only a rudimentary education and *without* attending university. Moreover, she acknowledged the technological importance of Faraday's work but did not see him as having to choose between performing experiments on optical glass and seeking the laws of electromagnetism.

Sir George and Mrs Thatcher have thus employed images of Faraday for their own purposes. In this they are not original. Both during the latter half of his life and especially since his death in 1867 images of Faraday have been prominent and potent. However, those images have been contradictory and used for a variety of purposes. Three examples will suffice.

One image is encapsulated in the title of John Tyndall's *Faraday as a Discoverer* (1868). Faraday's success at scientific discovery has often been attributed to his (presumed) use of the inductive, experimental method. Thus this image has been deployed to make a methodological point.

Again, Faraday has often been seen as morally upright and Victorians frequently praised him for his humility, honesty and virtue. Indeed his attractive personality and moral virtues have been used to account for his success in science. Thus the Good has been linked with Truth.

A third image relates to his success as a science lecturer. This image has recently been reinforced by the Royal Society's concern to improve the public understanding of science. A new prize has therefore been instituted to reward those who make notable contributions to this area: the Faraday Prize.

In their uses of Faraday, Sir George and Mrs Thatcher stand in a long tradition and both have drawn on the Victorian image bank. Would Faraday himself have endorsed either of them? He certainly recognized the importance of the human spirit but he would not have seen his life in terms of worldly success. He was a member of a small Christian sect, known as the Sandemanians, who sought to live their

lives according to the Bible and in imitation of Christ. Worldly success was not on their agenda and Faraday rejected all civil honours from Britain; he believed that they were tarnished by association with party politics and were not awarded for merit. He remained plain Mr Faraday and a servant of the Royal Institution, eschewing power and personal fortune.

It has sometimes been claimed that Faraday was a Tory, but although some of his attitudes were distinctly conservative, he strove to remain outside (if not above) party politics. This stance was shared by other Sandemanians who also insisted that the Bible required them to behave as loyal, law-abiding citizens. Thus out of a sense of duty Faraday frequently engaged in projects defined by the government and its agencies—for example he spent much time trying to improve lighthouse illuminants for Trinity House and he also experimented with different stone preservatives for use on the Houses of Parliament. Likewise, as Sir George pointed out, he felt duty-bound to enhance the Royal Institution and its finances.

Yet it is hard to believe that this loyal citizen would have supported Mrs Thatcher's policy towards science. He believed that, as part of God's providence, the laws of nature were fashioned to improve our lot. Science should be used for the good of mankind—he instanced such socially important science-based developments as the steam engine and the telegraph—but he was deeply opposed to the spirit of capitalism and the cult of affluence. Thus while knowledge of nature (pure science) should be used to assist mankind, it should not be exploited by those intent on lining their own pockets. The scientist and engineer deserved moderate rewards from applying their skills: for Faraday, however, they should not become rich through science, or else science would be corrupted.

Faraday would have viewed today's entrepreneurs and financial speculators as highly immoral. For him, as for other Sandemanians, the crucial text was Matthew 6:19—'Lay not up for yourselves treasures upon earth'. He would also have rejected the views Mrs Thatcher expressed recently before the General Assembly of the Church of Scotland. Faraday actively practised Biblical precepts: he prayed with the poor members of the Sandemanian community, loved them and supported them both spiritually and materially. On these points, at least, Mrs Thatcher has the wrong hero. □

Geoffrey Cantor is Gifford Fellow in the Department of Modern History, University of Glasgow, Glasgow G12 8QQ, UK.

Conversion of the sceptics

A.R. Gardner-Medwin

SIR George Porter's defence of science driven by curiosity, over that directed to commercial goals, will have boosted the morale of the beleaguered scientific community in Britain. But how much should sceptics as to the value of curiosity-based research be influenced by the example of Faraday's success, or by the systematic work of Comroe and Dripps¹, showing that 41 per cent of key papers leading to a set of clinical advances were not concerned at all with clinical issues? The answer depends critically on how much scientific effort goes into directed and undirected research: information that largely we don't have and that those responsible for decisions may dangerously misjudge.

For simplicity, consolidate the two sides of the debate into two hypotheses HU and HD, according to which the number of advances per funding unit for research that is undirected (U) and directed (D) are in the ratios 2:1 and 1:2. Take the fraction of funding that is undirected to be u . Then the probability that an advance selected at random will prove to be of type U can be readily calculated for each hypothesis:

$$P(U:HU) = 2u/(u+1) \text{ (given HU)}$$

$$P(U:HD) = u/(2-u) \text{ (given HD)}$$

The theory of evidence² shows that the log ratio of these quantities is the correct measure of the *weight of evidence* for HU when examples are type U

$$w(U) = \log((4-2u)/(u+1))$$

The corresponding weight for examples of type D is the negative quantity

$$w(D) = \log((1-0.5u)/(u+1))$$

Weights defined in this way add linearly for independent observations. They are the correct increment to $\log(p/(1-p))$, where p is the observer's perceived probability of the correctness of HU. They are thus a good measure of the influence that advances from U and D science should have on policy makers.

The weight of evidence in favour of HU when there are type-U advances (Table 1(b)), is highly dependent on u , the fraction of funding that is undirected. This is extremely important, because u is a parameter about which most of us, scientists and government alike, are very vague. Our first priority should be to clarify it. Consider the risks otherwise. Suppose we are dealing with sceptics who currently believe in HD but are amenable to rational persuasion on the basis of the evidence. If they happen to believe that u is much higher than it really is, then they will grossly underestimate the evidence (by a factor of five if they take $u=0.9$ instead of $u=0.5$). What is more, they will grossly overestimate the benefits they would expect to accrue, on their own hypothesis

HD, from abolition of undirected research (Table 1(c)). Our efforts to persuade them may have much less effect than they should, simply through ignorance of the value of u rather than any lack of rationality.

Ignorance is a very real factor in the debate. A recent visitor to this college from Whitehall revealed, or so it seemed to us, a surprising lack of appreciation of how science funding actually works. He was not aware that in order to support

Table 1 Evidence and implications for hypotheses favouring U or D funding

	Fraction of undirected funding (u)				
	.10	.25	.50	.75	.90
(a) $P(U:HU)$	.18	.40	.67	.86	.95
$P(U:HD)$	.05	.14	.33	.60	.82
(b) $w(U)$	.54	.45	.30	.15	.06
$w(D)$	-.06	-.15	-.30	-.45	-.54
(c) Benefit					
$(u=0:HU)$	-9%	-20%	-33%	-43%	-47%
Benefit					
$(u=0:HD)$	5%	14%	33%	60%	82%

(a) The conditional probabilities P of a randomly chosen scientific advance proving to have been Type-U, given HU or HD; (b) the weights of evidence (units: $\log_{10}$ or 'bel' (ref. 1)) in favour of HU when a success is found to be undirected ($w(U)$) or directed ($w(D)$); (c) the benefit (percentage change in success rate) expected from abolishing Type-U funding (setting $u=0$), given HU or HD.

their research, people must regularly make detailed applications for grants to research councils and the like. He thought that adequate support was generally available through the University Grants Committee (UGC), in the way that perhaps it was for many scientists in the 1960s. Because UGC support at one time represented the purest form of undirected funding, available at the discretion of a Head of Department who would usually see things much the same way as one did oneself, we must perhaps count this 'man from the ministry' as having been, at least before these discussions, a $u=0.9$ man.

Even Comroe and Dripps, in their carefully researched survey, appear to overlook the importance of the relative sums devoted to different types of funding. In their conclusion (quoted also by the House of Lords Select Committee in a recent report³) they say that "basic research pays off in terms of key discoveries almost twice as handsomely as other types of research and development". What the data actually showed was that 61 per cent of the discoveries had arisen from basic (that is, mechanism-orientated) research. The advantage in terms of discoveries per pound or dollar will have been more than twice if the fraction of funding devoted to basic research was less than about 45 per cent, and less otherwise.

The relevant funding statistics are not readily available, but for comparison the breakdown of the principal medical research funding in Britain by source in 1985-1986 was: Medical Research Council (MRC) 16 per cent, Department of Health and Social Security 2 per cent, charities 15 per cent, pharmaceutical industry 66 per cent³. It is hard to see how basic research funding in Britain (for which the main source is the MRC) could be as high as 45 per cent.

The terms 'basic', 'non-clinical', 'undirected' and 'curiosity-based' all have different definitions and boundaries. The last two categories are probably the smallest, and their funding fractions are easily overestimated. Within basic research in universities much funding goes on salaries to people who are either not free enough (as someone's assistant) or not temperamentally inclined to pursue their own curiosity-based research. Though the project may originate from a chief investigator's curiosity, or at least the curiosity of some years previously, it may also have been tailored to correspond to what a Research Council is known to favour (that is, directed research). Even project leaders with tenured jobs frequently mortgage their liberty through research contracts or grants, in the manner of Faraday, to the extent that if they take these contracts seriously they can hardly conduct curiosity-based research. The core of truly undirected funding in Britain has probably steadily diminished along with the declining adequacy of UGC funds for research, and is probably at present a small fraction.

A sceptic may be converted by the undermining of his logic as well as the presenting of evidence. Those who support directed funding may base their belief on what seems in general a highly rational principle: when you spend money you ought to know what you will get for it and why it will be of benefit. Scientific success depends on a combination of ideas and facts. On the whole, if you want facts in science you can buy them. Directed funding is the best way of buying them. It is the nature of ideas, however, that you don't know what they are until you have them. You can provide an environment to nurture them and as a funding agency you can solicit them, but you cannot generally direct their production. It is ideas that benefit most from curiosity-based research with flexible funding. The reasons for this are neither very odd nor in the least inconsistent with the British government's monetarist principles. □

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A.R. Gardner-Medwin is in the Department of Physiology, University College London, Gower Street, London WC1E 6BT, UK.

Deep rifts as sources for alkaline intraplate magmatism in eastern North America

Stephen Paul Phipps

Geology Department, The University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA

Intracontinental magmatism in eastern North America does not exhibit the regular time progression associated with mantle plume activity. Instead, the magmatism is sporadic but locally persistent, suggesting that the magmas rise from a mantle source that drifts with the continental plate. A spatial association with ancient rifts further suggests that this source is 'fertile', melt-generating material emplaced in the mantle lithosphere during the early stages of rifting.

ALTHOUGH most magmatism occurs at plate margins, small-scale mantle-derived magmatism is widespread in plate interiors. Igneous rocks within plates include: alkali olivine basalts; kimberlites, alnoites and mica peridotites (here referred to as 'kimberlitic rocks')¹; and ultrapotassic rocks and carbonatites. Phase-petrological studies indicate that many of these originated in the mantle, at least 200 km deep in the case of diamond-bearing kimberlites¹⁻³. Intraplate magmas are typically enriched in 'incompatible' elements (light-rare-earth, large-ion-lithophile and high-field-strength elements, and volatiles)². Here I show that some intraplate magmatic belts in eastern North America are spatially associated with rifted zones, and discuss possible causes for this association.

Mid-plate magmatism

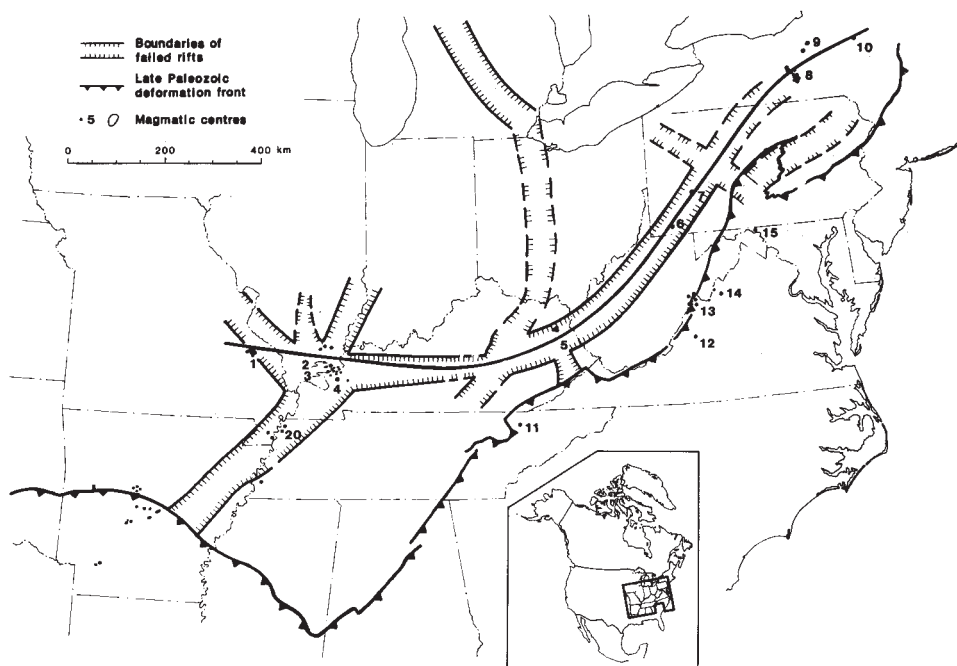
Figures 1 and 2 show the locations of mantle-derived, mid-plate igneous rocks in eastern North America, including alkali olivine basalts and other alkaline, carbonatitic and kimberlitic rocks. I have excluded the abundant, dominantly quartz-tholeiitic, late Triassic and early Jurassic basalt flows and sills erupted in graben and half-graben ('Triassic rift basins') along the eastern and southern coasts of the United States. These rocks represent active spreading-centre magmatism; thus they are plate-margin lavas (related to the opening of the Atlantic Ocean and Gulf of Mexico), as opposed to intraplate magmas.

In contrast magmatism along the late Palaeozoic deformation front of the Appalachian and Ouachita mountains (Figs 1 and 2) is mid-plate magmatism. Well dated magmatic centres along these trends are mainly of middle and late Cretaceous age, and almost all are younger than late Jurassic and older than Eocene. At these times, the adjoining oceans had opened sufficiently to place the magmatic centres well within the North American plate, far from the spreading ridge. The late Palaeozoic centres at Norris, Tennessee, and Clear Springs, Maryland, may have formed during Alleghenian convergence, but are hundreds of kilometers from any suture that might represent a contemporary plate boundary.

One igneous belt or trend extends along the 38th Parallel Lineament⁴ from Missouri to Kentucky and consists of kimberlitic rocks of Devonian and Permian age (Table 1 and Fig. 1). Igneous centres in south-west Pennsylvania and central New York (Numbers 6-10, Table 1 and Fig. 1) that may form a north-eastern extension of the 38th Parallel trend are of Jurassic age.

Another trend extends along the northern edge of the Gulf coastal plain, with a branch reaching north-eastward into the Mississippi embayment (Table 2 and Fig. 2). The igneous centres along this trend consist of alkali olivine basalt, nepheline syenite and phonolite, carbonatite, and kimberlitic rocks, mainly of late Mesozoic age.

Fig. 1 Positions of known alkalic and kimberlitic igneous centres in the east-central US, as described in Table 1, showing traces of failed rifts. The heavy line through the intracontinental failed rifts is the best-fit plume track through the igneous centres.



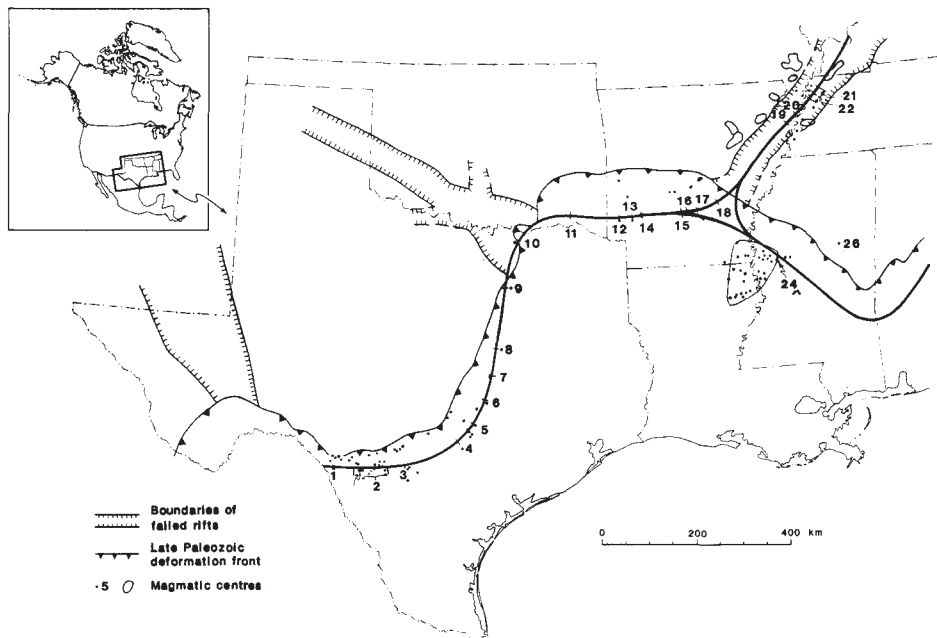


Fig. 2 Positions of known alkalic and kimberlitic igneous centres in the south-central US, as described in Table 2, showing traces of failed rifts. Igneous centres associated with the Rio Grande rift province in west Texas are not shown. Outlines of large plutons in the Reelfoot Rift/Mississippi Embayment region (the vicinity of localities 19–22) are from Hildenbrand and others⁵⁹. The heavy line is as in Fig. 1.

Table 1 Ages and lithologies of igneous centres in the east-central United States

Number on Fig. 1	Location	Rock types*	Age (Myr) and dating method†	Comment	Reference
38th Parallel province					
1	Avon, MO and vicinity	Kimb., alno.	383–399 (ear.–mid-Dev.) Rb/Sr-B, K/Ar-B	79 diatremes; associated dykes	9,41
2	Hicks Dome, IL	Kimb.	263–287 (late Perm.) K/Ar-B, H		41
3	Rosiclare, IL	Mica perid.	274 K/Ar-B; 260 Rb/Sr-B (ear.–mid-Perm.)	Sill in fluorspar district	9,41
4	Levias, KY	Mica perid.	257 K/Ar-B; 279 Rb/Sr-B (ear.–mid-Perm.)	Dyke in fluorspar district	9,41
5	Elliott Co., KY Ison Cr.	Kimb.	275–285 K/Ar-B; 257 Rb/Sr-B (ear.–mid-Perm.)		9,41
North-east extension					
6	Masontown, PA	Kimb.	post-Penn.		7,9
7	Dixonville, PA	Kimb.	185 K/Ar (mid-Jur.)		7,9
8	Ithaca, NY	Kimb.	145 ± 7 K/Ar-P; 136 ± 8 Rb/Sr-P; 120–140		41,42
9	Syracuse, NY	Kimb.	Post-Silur.		43,44,45
10	Manheim, NY	Kimb.	153 ± 8 K/Ar-B; 118 ± 15, 146 ± 8 Rb/Sr-B		9,41
Appalachian province					
11	Norris Lake, TN	Kimb.	Miss. to Perm. ?		9
12–14	Staunton, VA and vicinity	Ne-syen.	148–156 K/Ar-B (late Jur.); other centres Jur.–Eoc.	District also has teschen., picrite, mica perid., kimb.	9,12,41
15	Clear springs, MD	Kimb.			7

* AOB, alkali olivine basalt; alno., alnoite; bent., Bentonite; biot. anal., biotite analcite; carb., carbonatite; fourch., fourchite; ijol., ijolite; kimb., kimberlite or kimberlitic rocks; mica perid., mica peridotite; moniq., moniquite; ne-syen, nepheline syenite; ol-thol., olivine tholeiite; phon., phonolite; syen. syenite; teschen., teschenite.

† Mineral ages: B, biotite; H, hornblende; P, phlogopite; W, whole rock; Z, zircon.

A third trend follows the Appalachian deformation front north-eastward from Tennessee as far as Vermont and Quebec (Fig. 1). The igneous rocks are mostly kimberlitic but include nepheline syenites and alkali olivine basalts. The rocks are mainly late Jurassic and Cretaceous in age.

Rifting and intraplate magmatism

Figures 1 and 2 show that there is a strong correlation between the 38th Parallel and Gulf Coast magmatic trends, and late Precambrian–Phanerozoic rift zones. Localities 1–5 in Fig. 1 lie within the Rough Creek Graben–Rome Trough system of failed rifts, well known from seismic and well data, which extends from eastern Missouri through Kentucky, West Virginia, and probably south-western Pennsylvania⁵, and is associated with the successful rifts that opened to form the proto-Atlantic (Iapetus) Ocean and the proto-Gulf of Mexico⁵. Igneous centres 6 and 7 in Pennsylvania overlie the northeastern end of the Rome Trough (Olin basin of Wagner⁶). The centres in central New York are collinear with the Rome Trough and overlie a suggested early Palaeozoic basement normal-fault zone⁷. In this area, gentle anticlines above a Silurian salt detachment may occur over thrust-ramps or splays that developed because of detachment-horizon facies changes controlled by early Palaeozoic basement normal faults.

Heyl⁴ and Zartman⁸ associated other igneous centres with the 38th Parallel lineament, but these all overlie rifts. Cretaceous kimberlites in Kansas⁹ overlie the southern segment of the Proterozoic central North American rift system, and its diffuse southern end¹⁰; Jurassic picrites, and nepheline-syenites and Eocene andesites in northwestern Virginia and eastern West Virginia^{11,12} are part of the Appalachian trend defined here.

Another arm of the late Precambrian–Cambrian failed rift system, the Reelfoot Rift, extends southwest from southern Illinois along the lower Mississippi Valley beneath a weakly developed Mesozoic aulacogen (Fig. 2)^{13,14}. Localities 19–22 of the Gulf Coast trend overlie this rift, which is well known from geophysical studies and from drilling.

In southern Arkansas, and possibly in central Pennsylvania and eastern Tennessee, the extensive late Precambrian–Cambrian failed rift system intersects the successful Iapetus–proto-Gulf rift system⁵ that opened at the same time. The Appalachian igneous belt, and much of the Gulf Coast belt, probably follow the landward edge of this successful rift system.

The coincidence of the Appalachian and Gulf Coast trends with the front of the Pennsylvanian Marathon–Ouachita–

Table 2 Ages and lithologies of igneous centres in Gulf Coast Province

Number on map (Fig. 2)	Location	Rock types	Age (Myr) dating method	Comment	Reference	Number on map (Fig. 2)	Location	Rock types	Age (Myr) dating method	Comment	Reference
1	Kinney Co., TX	AOB	75 ± 3; 82 ± 4; Camp.-Maastr. (late Cret.)	West end of Balcones province; >100 small centres	46,47,49	14	Murfreesboro, AR	Kimb.	108.5 ± 3, K/Ar-P (Alb., mid-Cret.)	Four pipes	8,9
2	Uvalde Co., TX	AOB	65-84, K/Ar-W; Camp.-Maastr. (late Cret.)	Balcones prov.	46,47,49,51	15	Potash Sulphur Sprs. Garland Co., AR	Syen.	100 ± 2 U/Pb-Z, Pb/Pb-Z, Pb/Th-Z (Alb. mid-Cret.)		53
2a	Uvalde Co., TX (West Prong Fork)	AOB	Cenomanian (mid. Cret.) (stratigraphic)	Balcones prov.	47	16	Magnet Cove, AR	Ne-syen.; carb.; ijol.	97-99, K/Ar-B; 102-104, K/Ar-W; 99 ± 8, Rb/Sr-B (Alb.-Cen., mid-Cret.)	Potash Sulphur Sprs. intrusion is 10 km west	41,49
3	Frio Co., TX	AOB	77 ± 4, K/Ar-W (Campanian, late Cret.)	Balcones prov.	49	17	Cleveland Co., AR	Kimb.	Cret.?	In well	54
4	Guadalupe Co., TX	AOB	83 ± 3, K/Ar-W (Campanian, late Cret.)	Balcones prov.	49	18	Granite Mtn, AR	Ne-syen.	89-93 ± 5, K/Ar-B; 86 ± 3, Rb/Sr-B (Cen.-San., mid-Cret.)		41
5	Travis Co., TX (Pilot Knob)	AOB	80 ± 3, 83 ± 4, K/Ar-W (Campanian, late Cret.)	Balcones prov.	49,50	19	Lion-Bateman #1, Tipton Co., SW TN	Ne-syen.	Late Cret.?		55
6	Williamson Co., TX (Thrall and Chapman fields)	AOB	Campanian, late Cret. (stratigraphic)			20	Pure Robroy-McGregor #1 Lauderdale Co., W. Tennessee	Ne-syen.	Late Cret.?		55
7	Bell Co., TX	Bent.	2nd half Turonian (stratigraphic)	Poor data	52	21	Pemiscot Co., Mo, and Lake Co., TN	Moniq., fourch.	Late Cret.?		55
8	McLennan Co., TX	Bent.	2nd half Turonian (stratigraphic)	Poor data	52	22	A. E. Markham #1 (Lake Co., TN)	Kimb.	272 ± 8, K/Ar-B (ear. Perm.)		8
9	Dallas Co., TX	Bent.	2nd half Turonian (stratigraphic)	Poor data	52	23	Monroe Uplift, N.-central LA	AOB, Ne-syen., phon.	Late Cret.?		55
10	Grayson Co., TX	Bent.	2nd half Turonian (stratigraphic)	Poor data	52	24	Union C.B. Box #1 Humphreys Co., MS	Phon., biot. anal.	80 ± 3 K/Ar-W; 93 ± 3 K/Ar-B		56
11	Lamar and Red River Cos, TX	Bent.	2nd half Turonian (stratigraphic)	Poor data	52	25	Jackson Dome, MS	Ne-syen., phon.	Late Cret.?		54,55
12	Sevier Co., Ar ('Lockesburg volcano')	Phon.	Late Cenomanian-mid-Turonian (mid. Cret.) (stratigraphic)	Pure vitric-crystal tuff, phon. cobble conglomerate	52	26	Pan American #1 USA-Tombigbee Forest Choctaw Co., MS	Ol-thol.	139 K/Ar-W		57
13	Howard Co., Ar ('Nashville volcano')	Phon.	Late Cenomanian-mid-Turonian (mid-Cret.) (stratigraphic)	Pure vitric-crystal tuff, phon. cobble conglomerate	52	27	Scott Co., AR	Kimb.	Cret.?	Two dykes	58

Appalachian deformed belt is striking (Figs 1 and 2). Petrological, stratigraphic, structural, and seismic studies have shown that this deformed belt lies along the late Precambrian-early Palaeozoic rifted continental margin that bordered Iapetus and the proto-Gulf. Allochthonous, thick, late Precambrian volcanic and clastic rocks in the Blue Ridge probably represent large, late Precambrian rift basins^{15,17}. Seismic reflection profiles suggest that similar but autochthonous basins underlie the Blue Ridge and Inner Piedmont¹⁸⁻²¹. Basement-cutting normal faults may control the sites of thrust-ramp complexes 100 km to the north-west in the Appalachian Valley and Ridge and Plateau provinces, by controlling facies changes in the rift- and drift-stage sedimentary rocks²²⁻²⁴. Other ramp complexes may have formed as thrusts were deflected upwards over basement buttresses formed during rift-stage normal faulting^{22,26}. Thus, the northward and westward limits of Appalachian crustal structures may approximate the limit of rift-modified continental lithosphere. This structural boundary has been repeatedly reactivated for over 600 million years (Myr), most recently during the

Mesozoic rifting that opened the modern Atlantic and Gulf, and thus its history is complex^{27,28}. The association of Mississippian to Tertiary mid-plate volcanism with the ancient rifted continental margin, however, seems clear.

Other rifts shown in Figs 1 and 2 are suggested by various plate-tectonic, stratigraphic and structural data. For instance, the failed rift extending north-west from western Virginia to join the Rome Trough (Fig. 1) is suggested by Rankin's¹⁵ analysis, and corresponds to a deep, faulted basement trough filled with Cambrian clastics²⁹. The failed rift shown extending north-west through Pennsylvania is also suggested by Rankin's¹⁵ analysis, and may correspond with known basement structural zones^{30,31}. The rift extending into northeastern Pennsylvania is suggested by the Scranton gravity high^{5,32}.

Mantle plumes and rifts

Many continental intraplate magmas have been explained as tracks left on the moving continental lithosphere as it passes above convective plumes in the mantle asthenosphere. The mag-

Fig. 3 Age progressions along the 38th parallel magmatic trend versus distance. The x-axis represents distance from west end of heavy line in Fig. 1. The locality numbers just below the abscissa are described in Fig. 1 and Table 1. Points represent radiometric ages, with appropriate error bars; heavy lines with higher error bars represent multiple ages from a single locality; error bars without points represent stratigraphically determined age ranges. Dashed line represents boundary between the 38th parallel trend proper and its north-east extension.

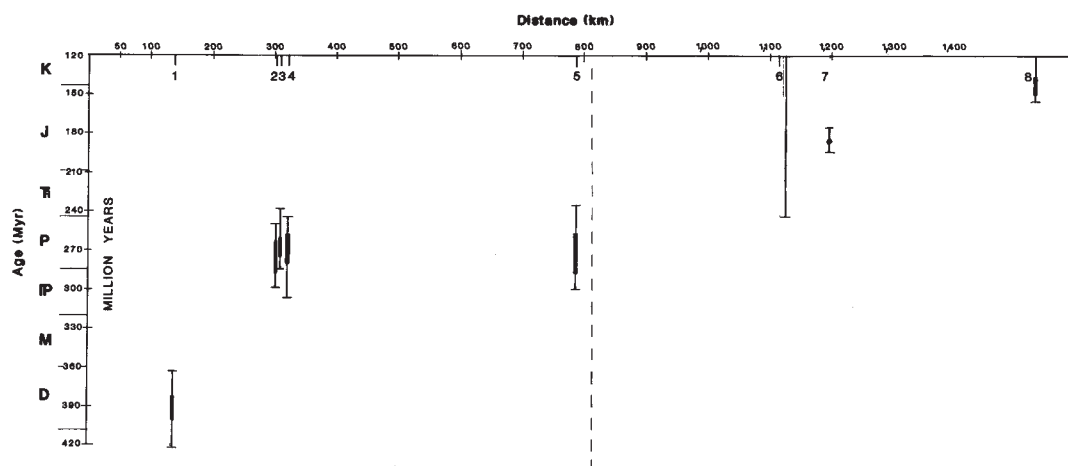
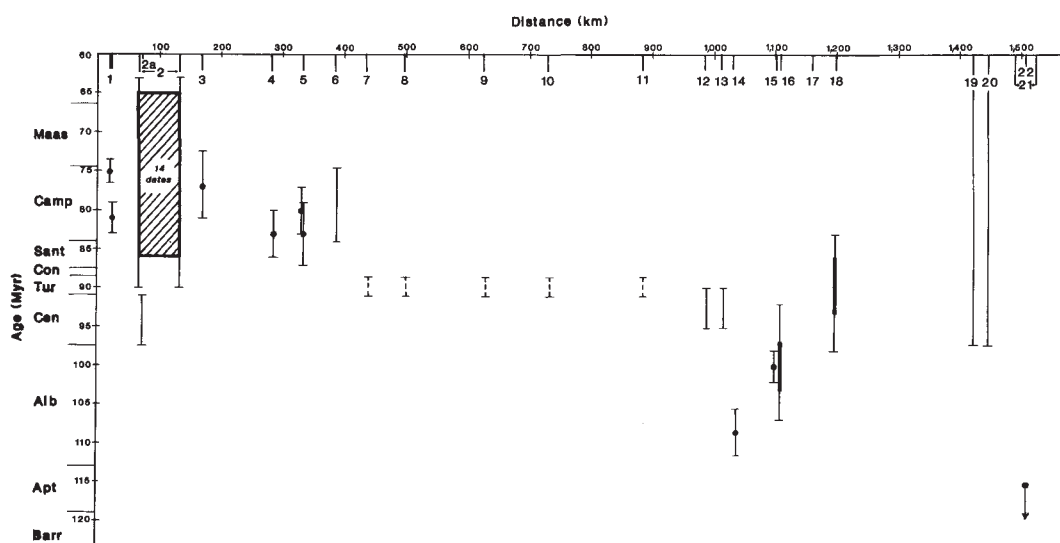


Fig. 4 Age progressions along the Gulf Coast magmatic trend versus distance. The x-axis represents distance from west end of heavy line in Fig. 2, following the northeastern branch up the Reelfoot Rift/Mississippi Embayment to localities 19-22. Locality numbers below abscissa are described in Fig. 2 and Table 2. Ages are shown as in Fig. 3. Hatched area represents range of 14 dates from centres in Uvalde County, Texas. Dashed error bars represent stratigraphic ages of bentonites, data of uncertain significance.



matism shown on Figs 1 and 2, however, cannot be explained by this mechanism. During the 50–300 Myr between rifting and magmatism, the continent would have moved 1,000–10,000 km away from any rift-age plumes. It seems an impossible coincidence that the tracks of much younger plumes should follow old rifts so closely. Furthermore, the magmatic belts are not linear, but sinuous, and the Gulf Coast belt branches, a geometry not permitted by the plume hypothesis.

Moreover, the times of magmatism in the belts do not progress in any regular manner. If an attempt is made to fit a straight line through the data for the 38th Parallel trend, critical data must be missed by at least 50–100 Myr (Fig. 3). A line forced through the data for the Gulf Coast trend implies late Cretaceous north-eastward movement of North America, a result incompatible with its well documented westward motion (Fig. 4)³³.

Rather, magmatism in each belt continued sporadically for long time-spans, from Devonian until mid-Permian time in the case of the 38th Parallel trend, or even until the Cretaceous if the trend's north-east extension is included. Even in some small areas—for instance, Uvalde County in West Texas (locality 2 in Figs 2 and 4), the upper Mississippi embayment, and north-western Virginia—magmatism lasted 30, 170 and 100 Myr respectively. Thus, these magmatic belts cannot be explained as simple plume tracks.

Causes of intraplate magmatism

The strong spatial association of mantle-derived magmatism

and lithospheric features (rifts), and the extended duration of magmatism in some locations, suggest that the igneous centres are related to long-lived, sub-rift features that are drifting with the North American continent. One possibility is that magmas rise from the asthenosphere to the surface along unhealed, lithosphere-cutting fractures beneath the rifts³⁴. In this model, magmatism occurs when the continent moves over 'melt-fertile' regions in the asthenosphere.

Several lines of evidence suggest an alternative mechanism: the melt sources themselves may lie within the continental lithosphere, and may move with the continent. Seismological studies suggest that the continental lithosphere is at least 200 km thick in many areas³⁵, and may be as thick as 400 km³⁶. Geothermobarometry suggests that the Kentucky and Pennsylvania kimberlitic rocks came from depths of 125–175 km, and the New York ones from 75–100 km; the other mantle-derived magmas discussed here probably rose from depths of less than 200 km, approximately the maximum equilibration depth for diamond-bearing mantle xenoliths in kimberlites^{37–39}. Thus, all these magmas may have originated in the subcontinental mantle lithosphere.

I suggest that the magmas formed because melt-generating material was emplaced in the continental lithosphere during rifting. Zones of incomplete continental rifting are commonly marked by volcanism like that associated with plumes⁴⁰, and mantle plumes may act as the causes and loci of rifting in the overlying continental lithosphere⁴¹. If rifting fails, this incompat-

ible-enriched material will become part of the mantle 'keel' beneath the continental interior. If rifting succeeds, it may cool and become part of the 'keel' along the craton-ward (inboard) edge of the transition zone between continental and oceanic lithosphere. In either case, the plume-emplaced material will move with the continent.

From time to time, these lithospheric fertile regions may give rise to magmatism. This might occur either during times of plate reorganization (supercontinental assembly or fragmentation) when both the stress and thermal regimes in the lithosphere might be disturbed, especially along ancient rifts, or during times of globally increased convective and magmatic activity (manifested by rapid seafloor spreading and increased volcanism) as may have occurred during parts of the Cretaceous. Such processes may result in sporadic, long-lived magmatism above some ancient rifts, as described here. Thus, although the 38th Parallel, Gulf Coast and Appalachian magmatic trends cannot be explained simply as plume tracks, they may be related to plumes in a less direct way.

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The mechanism I propose reconciles the data suggesting that mantle plumes are important in causing oceanic intraplate magmatism⁶⁰ with other data showing that continental intraplate magmatism is controlled by lithospheric, rather than asthenospheric, anomalies⁶¹. Moreover, this mechanism explains two different aspects of continental intraplate magmatism, that which occurs early in rifting and that which occurs after rifting has ceased, by a single mechanism.

Many other failed rifts and passive continental margins are marked by intraplate magmatism, some of it persistent, that occurred long after rifting⁶¹⁻⁶³. Many of these magmas may rise from zones in the continental lithosphere that were enriched by mantle plumes.

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The human oestrogen receptor functions in yeast

D. Metzger, J. H. White & P. Chambon*

Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS, Unité 184 de Biologie Moléculaire et de Génie Génétique de l'INSERM, Institut de Chimie Biologique, Faculté de Médecine, 67085 Strasbourg, France

The human oestrogen receptor when expressed in yeast stimulates initiation of transcription in a strictly hormone dependent manner, as in mammalian cells indicating striking conservation of the underlying regulatory mechanisms.

GENE expression in eukaryotes is regulated at many levels. The initiation of transcription is controlled by many proteins in addition to those of the basic transcription apparatus^{1,2} and there is increasing evidence that these control mechanisms have been conserved amongst eukaryotes ranging from yeast to man³.

*To whom correspondence should be addressed.

Initiation from yeast promoters, like mammalian promoters, is controlled by TATA elements^{3,4}. Generally yeast promoters also contain upstream activating sequence (UAS) elements which function in an orientation and somewhat distance independent fashion, thereby displaying the characteristics of higher eukaryotic enhancer elements⁵. UAS elements are DNA binding sites for transcriptional regulatory proteins such as GAL4⁶ and

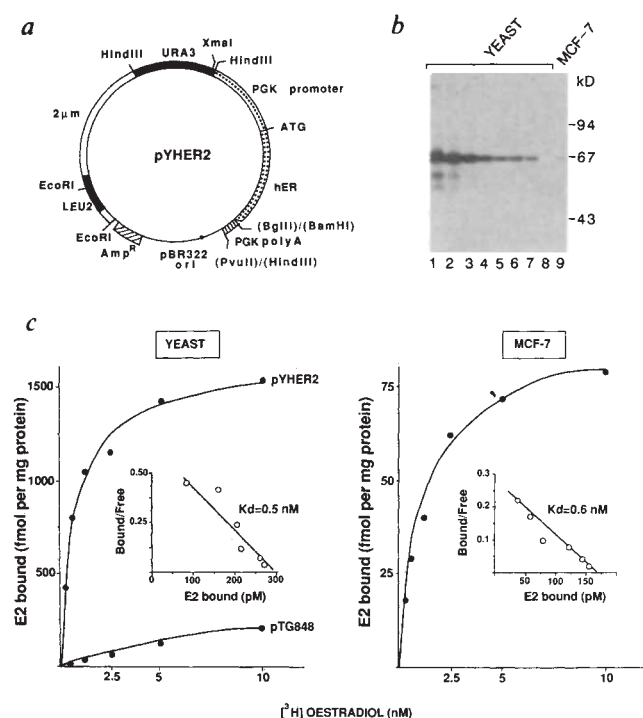


Fig. 1 Expression of the human oestrogen receptor in yeast. *a*, Vector pYHER2 which expresses the hER cDNA under control of the PGK promoter. *b*, Analysis of yHER expression by Western blot using monoclonal antibodies H222 and D75²⁷. Lanes 1 to 7 contain respectively, 20, 10, 5, 2.5, 1.25, 0.65 and 0.30 μg of whole cell extract from cells expressing pYHER2; lane 8, 20 μg extract from cells containing the control plasmid pTG848; lane 9, 20 μg of a whole cell extract from MCF-7 cells. *c*, The yHER has the same affinity for oestradiol as the MCF-7 hER. Hormone binding was measured using a dextran-coated charcoal assay²⁴. The inset shows a Scatchard plot of the data.

Methods. *a*, The parent plasmid pTG848 is an *E. coli*/yeast shuttle vector by (Transgene SA) which express genes in yeast under control of the PGK promoter^{25,26}. The first ATG of the PGK gene, converted to a BglII site by *in vitro* mutagenesis, permits the replacement of PGK-coding sequence by digestion with BglII. To create pYHER1, a 1.8 kilobase (kb) EcoRI fragment containing the entire hER cDNA was excised from pKCR2-ER²⁴, flanked with BamHI ends and inserted into the BglII site of pTG848. To construct pYHER2, the entire 5'-untranslated region of the hER gene of pYHER1 from the BglII/BamHI fusion to the first ATG of the coding sequence was deleted by site-directed mutagenesis, juxtaposing the PGK gene 5'-untranslated region immediately upstream of the hER coding sequence. This increased expression levels of hER protein ~fivefold (data not shown). *b*, The yeast strain TGY14 (*Mat a*, *ura3-251-373-328*, *leu2*, *pep4.3*) was transformed as described²⁴. Cells (10⁹) were harvested at an OD₆₀₀ of 1.0, washed once with phosphate buffered saline and disrupted with glass beads in 1 ml of hormone binding buffer²⁴. A series of twofold serial dilutions of extracts from cells expressing pYHER2 were made with extracts from cells expressing pTG848 from no dilution (lane 1) to a 64-fold dilution (lane 7). Each lane of the 10% polyacrylamide gel contained 20 μg of protein. Western blots were performed basically as described³⁷ using D75 and H222 monoclonal antibodies²⁷, rabbit anti-rat serum and ¹²⁵I-protein A. *c*, For hormone binding assays, yeast extracts were prepared as above and MCF-7 cell extracts as described²⁴.

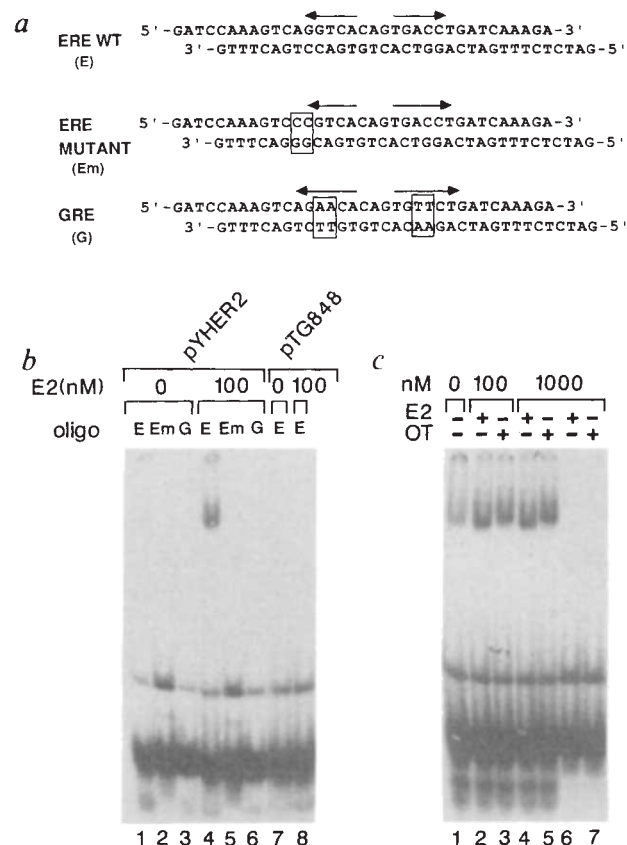


Fig. 2 Hormone-dependent binding by the receptor to its cognate DNA element. *a*, Sequence of the three 35 bp oligonucleotides used for gel retardation assays. The wild-type oligonucleotide (ERE WT) contains sequences from -310 to -341 upstream of the *Xenopus laevis* vitellogenin A2 gene²⁹, containing the 13 bp palindromic ERE sequence. The base changes made to create the mutant ERE sequence and the glucocorticoid responsive element (GRE, see ref. 31) are in boxes. *b*, Oestradiol stimulates specific DNA binding *in vitro*. Gel retardation assay with ³²P-labelled oligonucleotides and yeast whole cell extracts. The wild-type, ERE mutant and GRE oligonucleotides are indicated by E, EM and G, respectively. *c*, Comparison of the effects of oestradiol (E2) and hydroxy-tamoxifen (OT) on specific DNA binding by the receptor. Gel retardation assays were performed with wild-type ERE (lanes 1-5) or mutant ERE (lanes 6, 7) oligonucleotides.

Methods. While cell extracts of yeast were made using the same protocol described for hormone binding assays (Fig. 1) with the exception that cells were lysed in 20 mM HEPES (pH 7.9), 30 mM KCl, 0.5 mM dithiothreitol (DTT), 0.2 mM EDTA, 20% glycerol plus protease inhibitors. Oestradiol or *trans*-hydroxy-tamoxifen were added to the cleared lysates on ice as indicated and gel retardation assays³² were performed 2 h later. Gel retardation assays (20 μl) contained 10 μg of extract, 6 μg of poly-dIdC and 50,000-100,000 c.p.m. of oligonucleotide in 15 mM HEPES (pH 7.9), 80 mM KCl, 0.4 mM DTT, 0.2 mM EDTA, 4% glycerol. Mixtures were incubated 15 min on ice before addition of oligonucleotide and then 15 min at 23 °C before loading on 5% polyacrylamide gels equilibrated in 6.6 mM Tris, 3.3 mM sodium acetate (pH 7.5), 1 mM EDTA. Gels were run at 25 mA and dried before autoradiography.

GCN4⁷. Detailed molecular genetic analyses have shown that these proteins consist of discrete and separable DNA binding and transcription activating domains⁷⁻¹¹.

In vertebrates, nuclear receptors, the activity of which depends on the binding of specific ligands such as steroid hormones, vitamin D3, thyroid hormones or retinoic acid (refs 12-14 and refs therein) stimulate transcription by interacting with specific *cis*-acting sequences and display all the hallmarks of inducible

enhancer factors (refs 15-18 and refs therein). A series of studies, such as those with the oestrogen receptor, have shown that these receptor proteins comprise a highly conserved DNA binding domain (refs 14, 16-18 and refs therein) and an independent, less well conserved domain responsible for hormone binding and transcription activation (refs 14, 15, 17, 19, 20 and refs therein). Strikingly, chimaeric proteins consisting of the DNA binding domain of yeast GAL4 coupled to the hormone binding domains of either the human oestrogen (hER) or glucocorticoid receptor will stimulate transcription in HeLa cells when bound

to an UAS²¹. Conversely, the hER DNA binding domain mediates stimulation of transcription in HeLa cells by the activating regions of yeast GAL4 or GCN4 proteins²². Taken together, these results demonstrate that the hER and other nuclear receptors, as well as GAL4 and GCN4 proteins of yeast, consist of discrete and separable DNA binding and transcription activation functions. Furthermore, they show that the GAL4 DNA binding²¹ and transcription activation domains²², separately and in intact GAL4 protein^{22,23}, function in HeLa cells, revealing conservation of the regulatory mechanisms that control transcription initiation.

To further investigate these striking parallels, we have expressed the hER in the yeast *Saccharomyces cerevisiae* and have analysed its hormone and DNA binding properties *in vitro*, and its ability to stimulate transcription *in vivo*. We demonstrate that the hER stimulates transcription in yeast in a strictly hormone dependent manner, indicating an amazing conservation of the molecular mechanisms underlying this activation across all eukaryotes.

Oestrogen receptor in yeast

The plasmid pYHER2 (Fig. 1a) which contains the hER complementary DNA²⁴ under control of the yeast *PGK* (phosphoglycerate kinase) promoter^{25,26}, was introduced into yeast. The hER protein of relative molecular mass (M_r) 65,000 (65K) was detected by Western blot analysis using monoclonal antibodies H222 and D75 (ref. 27). The levels of soluble receptor produced in yeast (yhER) are 100- to 200-fold higher than those produced in the human breast cancer cell line MCF-7 (Fig. 1b, compare lanes 1–7 with lane 9). The lower M_r bands observed at higher concentrations of receptor (lanes 1 and 2) are very probably proteolytic fragments, as no cross-reacting proteins were detected in a control yeast strain containing the parent vector pTG848 (Fig. 1b, lane 8).

The level of specific oestradiol binding activity (expressed per mg of soluble protein) in the strains harbouring pYHER2 appears to be 20-fold higher than that detected in whole cell extracts of MCF-7 cells, and many fold above the endogenous oestradiol binding activity found in yeast²⁸ (Fig. 1c). Furthermore, the affinity of yhER for oestradiol ($K_d = 0.5$ nM) is very close to that for the receptor expressed in MCF-7 cells ($K_d = 0.6$ nM). Thus, by these criteria, the yhER is indistinguishable from hER expressed in human cells. There is, however, a discrepancy of roughly five-fold in the relative levels of hER detected in extracts of yeast and MCF-7 cells by Western blots compared to the relative hormone binding activity in the two extracts (Fig. 1b and c). Western blots of yeast extracts made after hormone binding assays suggest that the apparent inability of roughly 80% of the hER protein made in yeast to bind oestradiol is not due to proteolysis of the receptor during the hormone binding assay (data not shown).

Hormone-dependent DNA binding

The oestrogen responsive element (ERE) which mediates ER-dependent trans-activation is a 13 base pair (bp) palindromic sequence containing 5 bp arms and a 3 bp spacer region^{18,29,30}. For DNA binding studies, we used a 35 bp oligonucleotide derived from the sequence –310 to –341 upstream of the *Xenopus laevis* vitellogenin A2 gene²⁹, containing the perfect ERE core palindrome 5'-GGTCACAGTGACC-3', and two control oligonucleotides (Fig. 2a, sequence changes in boxes). One was the ERE mutant *Em*, which has two contiguous base changes, one of which (in the 13 bp core sequence (G→C)) drastically reduces hER-mediated transcriptional activation in MCF-7 cells³⁰. The other oligonucleotide (G) contains the perfect palindromic responsive element 5'-AGAACACAGTGTCT-3' recognized by the glucocorticoid receptor *in vivo* (GRE) (ref. 31 and refs therein).

Specific binding of the hormone to these oligonucleotides was monitored by gel retardation assays³² (Fig. 2b). It is clear that

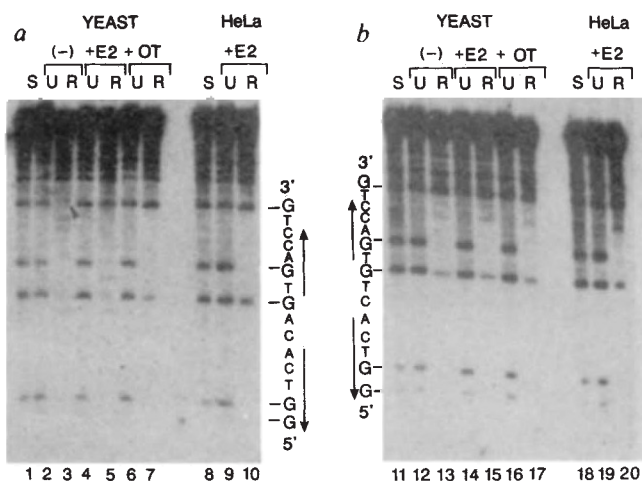


Fig. 3 Analysis of contacts between the oestrogen receptor and the ERE by DMS (dimethyl sulphoxide) methylation interference. Binding to both the top strand (panel a) and the bottom strand (panel b) of the ERE oligonucleotide (see Fig. 2a) was analysed. S is the starting DNA used for complex formation, U is the unretarded DNA and R is the retarded DNA. The sequence surrounding the ERE element is indicated, with G residues in the region highlighted.

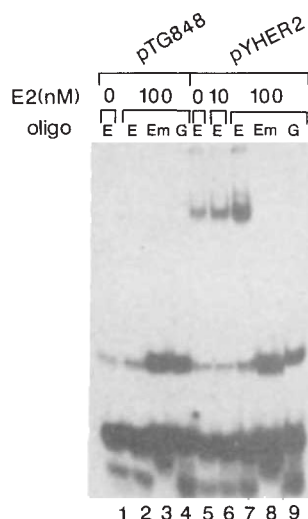
Methods. Yeast whole cell extracts were made as described (Fig. 2). HeLa nuclear extracts were made in the presence of 10 nM oestradiol as described⁵⁵ from cells transiently transfected with pKCR2-ER²⁴ and treated with 10 nM oestradiol 2 h before collecting. Incubations contained 10 µg of yeast extract or 6 µg of HeLa extract. Methylation interference was performed basically as described³⁴. ³²P-labelled DNA (2×10^6 c.p.m.) was incubated in 100 µl of 50 mM sodium cacodylate pH 8.0 at 20 °C for 4 min in the presence of 2 µl of DMS. Reactions were quenched by the addition of 200 µl of 300 mM sodium acetate, 1 M β-mercaptoethanol and 10 µg of transfer RNA, and then extracted with 200 µl of phenol/chloroform 1:1 and ethanol-precipitated. Gel retardation experiments were performed with 2×10^5 c.p.m. of oligonucleotide. Wet gels were autoradiographed and DNA was purified by electroelution. Cleavage of modified G residues with 1 M piperidine was as described³⁶. Purified products were resolved in 15% denaturing acrylamide gels and analysed by autoradiography.

a complex was formed on addition of hormone in extracts that contain the hER (pYHER2), but not in control (pTG848) extracts (compare lanes 4 and 8). Moreover the formation of the complex is markedly oestradiol-dependent (compare Fig. 2b, lane 1 and Fig. 2c, lane 1 with Fig. 2b, lane 4 and Fig. 2c, lane 2). Binding is specific to the ERE as no binding to the mutated ERE sequence (*Em*) or to the GRE (G) was observed, either in the absence (lanes 2 and 3) or in the presence of oestradiol (lanes 5 and 6). Interestingly, the anti-oestrogen hydroxy-tamoxifen (OT)³³ also stimulates specific DNA binding (Fig. 2c, compare lane 1 with lanes 3 and 5 and lane 7). The only discernible distinction between the complexes formed in the presence of OT and oestradiol was a reproducibly slower migration of the complex formed in the presence of OT.

Methylation interference³⁴ was used to analyse further the complexes formed with the ERE. The yeast complexes formed in the absence of hormone, and in the presence of oestradiol or OT, were compared with those formed in nuclear extracts of HeLa cells expressing the hER (in HeLa cells hER forms a complex with identical mobility to that formed with yhER; J.H.W. and V. Kumar, unpublished results). The interference patterns of the complexes formed in yeast extracts in the absence of hormone (Fig. 3, lanes 3 and 13), and in the presence of oestradiol (lanes 5 and 15) or OT (lanes 7 and 17) are essentially identical. Binding is completely prevented by methylation of any G residue on either strand within the palindrome (panels a and b), and by significantly inhibited modification of either G residue in the 3 bp spacer (the interference in binding in the

Fig. 4 Oestrogen receptor binds oestradiol *in vivo*. Gel retardation assays of yeast extracts from cells expressing the hER (pYHER2) or from cells harbouring the control plasmid pTG848. Wild-type ERE, ERE mutant and GRE oligonucleotides are indicated by E, EM and G, respectively.

Methods. Cells were treated with oestradiol (E2) in culture at the concentrations indicated, 2 h before collecting. Cells were washed twice and extracted as described (Fig. 2) in the absence of oestradiol. Gel retardation assays were performed immediately.



absence of hormone seen with methylation of the G residue outside the palindrome (lane 3, arrowhead) is not reproducible). Moreover, the interference patterns observed with yHER are essentially identical to those obtained with the receptor expressed in HeLa cells (lanes 10 and 20), indicating that similar contacts are made with the ERE. We conclude that the yHER binds specifically to its cognate DNA element in a hormone-dependent manner, and that the resulting complex is indistinguishable from that formed by the receptor expressed in HeLa cells.

Oestradiol activates transcription

To examine whether exogenous administration of oestradiol to yeast cultures would provide sufficient intracellular levels for ERE complex formation, oestradiol was added to log-phase cells 2 hours before they were collected. After extensive washing, the cells were processed without further added hormone and *in vivo* hormone binding to the receptor was assessed from the extent of specific DNA binding (Fig. 4). Administration of 10 nM oestradiol to the cultures stimulated specific binding of the hER only slightly (compare lanes 5 and 6), but 100 nM hormone (lane 7) stimulated binding to a similar extent to oestradiol added after lysis to extracts from cells that had been grown in its absence (Fig. 2). This binding is hER-specific (see control lanes 1–4 and compare lanes 7–9). Furthermore, if extracts of cells harbouring the parent vector pTG848, which had previously been treated with 100 nM hormone were mixed with extracts of cells containing the hER, but which had not been exposed to hormone *in vivo*, no stimulation of DNA binding was observed (data not shown). This indicates that the observed hormone binding actually occurred *in vivo* before cell lysis.

To test for hER-mediated activation of transcription in yeast, we used chimaeric constructs containing the coding sequence of the *Escherichia coli* β -galactosidase gene (*lacZ*) inserted downstream from the yeast *GAL1* promoter deleted for a sequence involved in glucose repression³⁵. In the control construct the *GAL1* UAS was retained, but in others it was replaced with ERE-containing fragments as indicated (Fig. 5a). These recombinants (Fig. 5a) also express the hER cDNA under control of the *PGK* promoter (see also Fig. 1a).

The *GAL1* promoter gives a low level of constitutive β -galactosidase expression which is not affected by the presence of the hER (compare pYAL with pYAL/HER, and pYERE1/HER with pYERE1/HERR which expresses the hER antisense RNA, Table 1). On addition of oestradiol, however, the promoter of pYERE1/HER is strongly induced (compare induction of pYAL/HER with that of pYERE1/HER), demonstrating that the hER is an oestrogen-inducible transcription activator in yeast. Several lines of evidence demonstrate that this action is through recognition of the ERE. First, no

Table 1 Hormone-dependent induction of β -galactosidase activity by the yHER

Plasmids	Glucose	Medium Galactose	Oestradiol	β -galactosidase activity (units)
YHER2	+	–	+	0
pYERE1/HER	+	–	–	60
pYERE1/HER	+	–	+	1,050
pYERE1/HERR	+	–	–	60
pYERE1/HERR	+	–	+	60
pYERE3/HER	+	–	–	60
pYERE3/HER	+	–	+	2,050
pYEREM/HER	+	–	–	60
pYEREM/HER	+	–	+	260
pYGAL/HER	+	–	–	180
pYGAL/HER	+	–	+	180
pYGAL	+	–	–	180
pYGAL	–	+	–	2,300

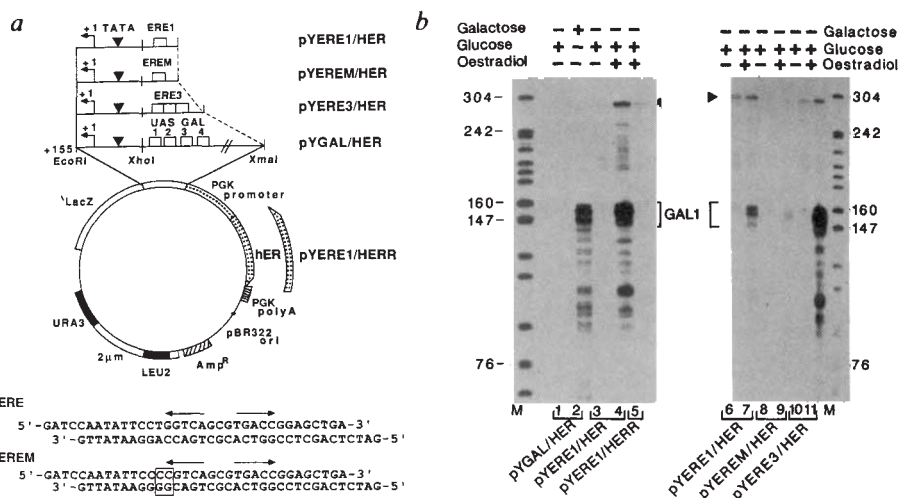
Yeasts harbouring the indicated plasmids were grown as described in Fig. 5b. Assays for β -galactosidase were performed as described³⁵.

induction by oestradiol is observed from the pYAL/HER recombinant which encodes the hER, but does not contain an ERE. Second, pYEREM/HER which contains a mutant ERE (*Em*) with a single point mutation in the ERE palindrome (Fig. 5a) that drastically reduces its activity in MCF-7 cells is ~20% as active upon induction as the wild-type derivative pYERE1/HER, perhaps reflecting the high level of hER expression in yeast. Conversely, the level of induction using the pYERE3/HER recombinant, which contains an ERE trimer, is twice that of the pYERE1/HER recombinant with only a single ERE. Indeed, the level of *lacZ* expression from the promoter of pYERE3/HER is sufficient to render yeast colonies containing this recombinant strongly blue in a β -galactosidase plate assay when treated with oestradiol (compare Fig. 6a and b)³⁵. Under the conditions described in Table 1, the *GAL4*-induced *GAL1* promoter in pYAL/HER generates approximately the same levels of β -galactosidase activity as oestradiol-induced pYERE1/HER and pYERE3/HER promoters. It is difficult to compare the relative activities of the pYAL and pYERE promoters, however, as the endogenous levels of *GAL4* protein are unlikely to saturate the pYAL/HER promoter on a multicopy plasmid³⁶.

To demonstrate that the activation induced by the hER plus oestradiol reflects stimulation of accurately initiated transcription, the 5' ends of the messenger RNAs were mapped using an RNase protection assay³⁷. The probe is a transcript of the *EcoRI* fragment containing the *GAL1* promoter region. As there are multiple initiation sites on the *GAL1* promoter³⁵, the probe is complementary to *GAL1* transcripts over a range of 154–165 nucleotides. The results shown in Fig. 5b are typical of several yeast RNA preparations. The size of the fragments protected by RNA prepared from cells expressing the galactose-induced pYAL/HER promoter (lane 2) are in close agreement with those expected for initiation from the *GAL1* promoter³⁵. Significantly, the results demonstrate that transcription from the pYERE1/HER promoter displays the same pattern of initiation sites as that of the pYAL/HER promoter (compare lanes 2 and 4) and the levels of transcription from both promoters appear very similar. Moreover, consistent with the results from the β -galactosidase assays, initiation from the pYERE1/HER promoter is strictly dependent on the presence of oestradiol (compare lanes 3 and 4) and on the presence of yHER (compare lanes 4 and 5). We note that there is not always a good correlation between the relative β -galactosidase activities detected in cell extracts and the corresponding RNA levels detected by RNase protection. The amount of RNA detected in extracts of cells containing pYERE3/HER is at least tenfold higher than in extracts of cells containing pYERE1/HER (compare lanes 7 and 11) or pYAL, whereas there is only a twofold difference

Fig. 5 The oestrogen receptor activates transcription *in vivo* in yeast. **a**, Vectors used to study hER-mediated activation of transcription. The vectors express (1) the hER cDNA (except for pYERE1/HERR, in which the hER cDNA was inserted in the reverse orientation) under control of the *PGK* promoter and (2) the *E. coli* *LacZ* gene under control of test promoters which contain ERE sequences, as indicated, upstream of the *GAL1* gene TATA box. The wild-type and mutant ERE sequences are presented at the bottom of the figure. **b**, Ribonuclease protection assays of RNA extracted from uninduced or induced cells containing plasmids as indicated, and probed with antisense *GAL1* RNA. Protected fragments derived from transcription initiated specifically at the *GAL1* initiation site are indicated by the brackets marked *GAL1*, whereas transcription initiated upstream of the region of homology between the probe and the promoters of the pYERE/HER series of recombinants is indicated by the arrowheads.

Methods. **a**, The ERE promoter-*lacZ* fusions were originally constructed in pLR1Δ20 (ref. 35) by replacing the *Xma*I-*Xho*I fragment upstream of the *GAL1* TATA box, which contains the four UAS elements recognized by GAL4, with a *Xma*I-*Xho*I fragment containing the ERE constructs indicated. Subsequently, an *Xma*I linker was inserted in the *Tth*111I site at the 3' end of the *lacZ* gene. The resulting *Xma*I fragment containing the ERE promoter-*lacZ* fusions was then inserted in the *Xma*I site of pTG848. To create the pYERE/HER series, the *Bam*HI fragment containing the hER cDNA was inserted in the *Bgl*II site downstream of the *PGK* promoter as for pYHER1 (Fig. 1). The ERE was cloned as a 35 bp oligonucleotide with *Bam*HI and *Bgl*II ends, containing sequences from -605 to -634 of the chicken vitellogenin II gene^{37,38}. The trimer of pYERE3/HER is a direct repeat of this sequence. The mutant sequence, EREM (changes in boxes), contains the G→C transversion that drastically reduces the activity of the ERE in MCF-7 cells³⁰. **b**, Yeast cultures were grown as described in Fig. 1 with either 1% glucose or 2% galactose as indicated. RNA extractions⁵⁹ and ribonuclease protection assays³⁷ were performed as described. For induction by oestradiol, 100 nM hormone was added to yeast cultures 4 h before collecting the cells. To create the probe, the *Eco*RI fragment from pLR1Δ20³⁵ containing the *GAL1* promoter region was cloned into Bluescribe M13⁺. The probe is a T7 RNA polymerase transcript. As there are several initiation sites in the *GAL1* promoter³⁵, the probe is complementary to *GAL1* RNA over a range of 154-165 nucleotides. Probe was incubated with 20 μg of RNA from each extract before ribonuclease treatment. The results presented are typical of several RNA preparations. Band intensities were quantitated by densitometry (see text).



in the relative β -galactosidase activities (Table 1). This observation raises the possibility that there may be a post-transcriptional step(s) which limits the production of β -galactosidase under conditions where the *lacZ* gene is expressed at very high levels.

Discussion

We have demonstrated here that the human oestrogen receptor stimulates accurate initiation of transcription in yeast through recognition of an ERE, and, as in mammalian cells, this is strictly dependent on the presence of oestradiol (Table 1 and Fig. 5). Thus, the basic molecular mechanisms of hormone-dependent transcription activation by the hER operate in yeast.

We have also shown that the ERE-binding properties of the hER produced in yeast (Fig. 2b and c and Fig. 4) or HeLa cells (V. Kumar and P.C., in preparation) are almost identical. Both are strongly stimulated by oestradiol and by the anti-oestrogen hydroxy-tamoxifen. The DNA binding observed *in vitro* is specific for the ERE as it is prevented both by a single point mutation which disrupts ERE-mediated transcription *in vivo*³⁰ and by substitution with a GRE^{31,38} (Figs 2b and 4). In addition, the results of methylation-interference experiments (Fig. 3) were essentially identical for the receptors expressed in both yeast and HeLa cells. The only difference was that the specific hormone-binding activity found in yeast extracts, is about five-fold lower than that expected from the relative amounts of hER detected in yeast and MCF-7 extracts (Fig. 1b and c). While we have no explanation for this observation, it raises the possibility that post-translational modifications may influence the activity of the receptor in yeast.

The hormone-dependent transcription stimulated in yeast cells from chimaeric promoters containing ERE's and the *GAL1* TATA box is initiated at the same sites as the wild-type yeast *GAL1* promoter (Table 1 and Fig. 5a and b). The yHER is clearly acting through recognition of an ERE as this induction depends on the presence of an ERE and is modulated by changes in the sequence (pYEREM/HER) or number (pYERE3/HER)

of EREs. There is a greater than tenfold difference in induction observed between a plasmid containing an ERE trimer and one containing an ERE monomer (Fig. 5b). This is reminiscent of the synergism previously observed in MCF-7 cells between pairs of mutant EREs¹⁸.

Induction of transcription by the yHER is strictly dependent on the presence of oestradiol (Table 1 and Fig. 5b). Previous studies using *GAL4*-hER chimaeras expressed in HeLa cells have shown that the hormone is required both for specific binding to the ERE²² and for induction of the transcription activation function²¹. The anti-hormone hydroxy-tamoxifen is able to promote ERE-specific binding, but not to induce the activation function²¹. It has thus been proposed that the hor-

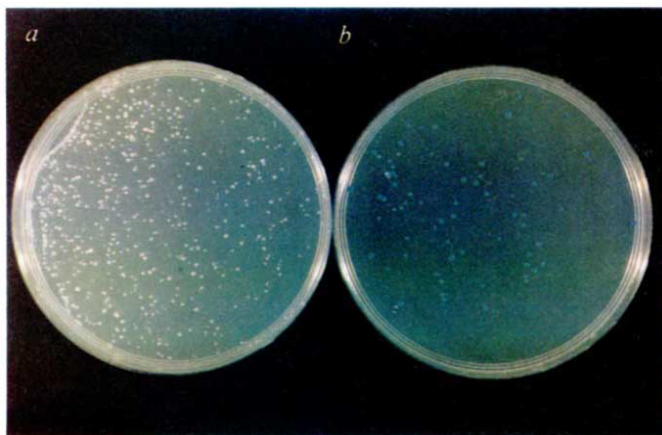


Fig. 6 Induction of β -galactosidase activity by the hER is dependent on oestradiol. Yeast containing pYERE3/HER were grown³⁵ on plates containing the chromogenic substrate 5-bromo-4-chloro-4-indolyl- β -D-galactoside (X-gal, 40 μg ml⁻¹) in the absence (a) or presence (b) of 1 μM oestradiol.

none (or the anti-hormone) is required for 'unmasking' of the DNA binding domain, which may be hidden by the 90K heat-shock protein (HSP90) found in receptor complexes unoccupied by hormone (see ref. 39 and refs therein). The ligand requirement for ERE binding in yeast suggests that a similar mechanism may operate here. As yeast produces both constitutive and inducible homologues of vertebrate HSP90^{40,41}, the 'masking' hypothesis is now amenable to both biochemical and genetic analysis. Although we have no direct evidence that induction of the transcription activation function of the hormone-binding domain of the yHER is oestradiol-dependent, there is no reason to believe that this should be different from the observations in HeLa cells.

The activating domain of the yeast transcriptional activator GAL4 functions in mammalian cells in a manner indistinguishable from that of mammalian enhancer factors^{22,23}. It is perhaps more surprising that a transcription activator required for specialized functions during vertebrate development, like the hER, is active in yeast. Recently, however, it has been shown that the putative transcriptional activator *c-fos*, when linked to the DNA binding domain of the bacterial *LexA* repressor, will function in yeast strains at promoters engineered to contain a *LexA* operator⁴². In addition, the DNA binding domains of the product of the avian oncogene *v-jun* and the yeast transcription activator GCN4 are functionally homologous⁴³ and the *c-jun* protein shares a common DNA-binding specificity with mammalian transcription factors AP1^{44,45} and *c-fos*⁴⁶, suggesting that these yeast, avian and mammalian transcription activators have a common progenitor. If enhancer factors of higher eukaryotes function by interacting with some component(s) of the transcription apparatus, as indicated by studies with the SV40 early promoter⁴⁷, the previous^{22,23} and present studies suggest that these interactions are conserved between yeast and man.

The yHER functions in the apparent absence of *cis*-acting elements other than the ERE and the *GAL1* TATA box (see Fig. 5a). This suggests that it interacts either with RNA polymerase B (II), its ancillary initiation factors or the TATA box factor. The primary structure of the largest subunit of eukaryote RNA polymerase B (II) is conserved from yeast to man (refs 48, 49 and refs therein). The TATA box factor also appears to be conserved as a HeLa cell *in vitro* transcription system which lacks this factor can be complemented by an activity present in yeast extracts⁵⁰. Given that the transcription activation regions of yeast *trans*-activators like GAL4 and GCN4 proteins and

that of the hER show no apparent homology, the interactions between these factors and the transcription apparatus cannot be very specific. A common activating element in these factors may simply be an acidic region^{51,52}, conceivably forming an amphipathic helix⁵² which is hidden in the hER. We cannot rule out the possibility, however, that the range of structures which activate transcription reflects a different mechanism, such as alteration of chromatin structure^{2,53}. Indeed, the conservation of the transcription apparatus may merely reflect strict structural requirements for initiation and elongation of RNA chains.

The production of functional hER in yeast opens new avenues for structure-function studies of this, and other, higher eukaryotic *trans*-activators, as well as for *in vitro* transcription studies aimed at elucidating the mechanism of *trans*-activation. In addition, yeast genetics can be used to further dissect the function of the DNA binding, hormone binding and transcription activating domains of the hER, and of course of other transcriptional factors. In this respect, it will be of interest to compare the effect of mutations on the function of the hER in yeast and in mammalian cells. Furthermore, our results suggest that yeast can be used for cloning the cDNAs of mammalian transcription *trans*-activating factors whose DNA binding sites have been characterized. Conversely, it should be possible to use yeast to identify the DNA-responsive elements of ligand-inducible mammalian *trans*-activators for which no target genes are known (refs 12 and 13, for example).

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Function of a yeast TATA element-binding protein in a mammalian transcription system

Stephen Buratowski*, Steven Hahn, Phillip A. Sharp* & Leonard Guarente

Department of Biology and * Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

Saccharomyces cerevisiae contains a protein which is functionally similar to the mammalian TATA element-binding transcription factor, TFIID. The yeast factor substitutes for TFIID in a mammalian RNA polymerase II *in vitro* transcription system, forms a stable preinitiation complex on the Adenovirus-2 major late promoter, and binds specifically to the TATA boxes of the viral promoter and the yeast CYC1 promoter. Interestingly, the yeast factor promotes initiation at a distance from the TATA element typical of a mammalian system.

RECENT reports suggest that the mechanism by which transcription by RNA polymerase II (pol II) is promoted is remarkably conserved in eukaryotes. It has been known for some time that the sequence of the large subunit of pol II has been highly conserved over eukaryotic evolution^{1,2}. More recently, several examples of sequence and functional similarity between mammalian and yeast transcriptional activators have been demonstrated. The yeast transcriptional activator GCN4 shares amino-acid sequence similarity and uses the same DNA-binding site as the protein product of the chicken oncogene *jun*^{3,4}, and its mammalian homologue *AP-1* (ref. 5). Transcriptional activators that bind to sites containing a CCAAT box are heteromeric complexes in both yeast and mammals⁶⁻⁸. Strikingly, the interactions between subunits of these complexes have been conserved, as formation of hybrid complexes by mixing a yeast subunit (either HAP2 or HAP3) with a mammalian subunit (either CP1A or CP1B, respectively) reconstitutes specific DNA binding⁹. As well as the DNA binding domains and subunit interactions, some aspects of the mechanism of transcription activation by upstream binding proteins have also been conserved. The yeast protein GAL4 stimulates transcription from a promoter containing the appropriate binding site in mammalian cells^{10,11}. Furthermore, the *fos* oncogene, a probable mammalian transcription factor, will activate transcription in yeast cells when fused to a gene containing a DNA-binding domain¹². The mechanism of transcription activation is believed to involve the interaction of an acidic domain on the upstream binding protein with some component of the basic transcription machinery (see ref. 13 for review).

Relatively little is known about the basic process responsible for initiation by RNA pol II in either mammalian or yeast cells. In both, initiation is typically dependent upon recognition of a TATA sequence element. In mammalian cells, the site of initiation is confined to a small region ~30 base pairs (bp) downstream of the TATA element¹⁴. In yeast, the start site can range over 60 to 120 bp downstream of the TATA box¹⁵⁻¹⁸. *In vitro* reactions containing extracts of mammalian^{19,20}, *Drosophila*²¹, and more recently, yeast cells²² have been shown to yield accurate transcription initiation by pol II. The simplest *in vitro* reaction is dependent solely on recognition of the TATA element in the DNA template. Chromatographic fractionation and biochemical analysis of mammalian cell extracts has revealed that four separate activities are required to reconstitute accurate initiation by purified pol II from a promoter containing a TATA element^{23,24}. These factors are: (1) a factor (TFIID, also known as DB and BTF1) that specifically recognizes and binds to the TATA element; (2) a protein (TFIIA, AB, STF) with a relative molecular mass (M_r) of ~19,000 (19K)²⁵ that when added with TFIID, facilitates formation of a stable preinitiation complex on the promoter^{26,27}; (3 and 4) two other factors (TFIIB, CB I, BTF3; and TFIIIE, CB II, BTF2) that presumably promote initiation through their interactions with each other and the poly-

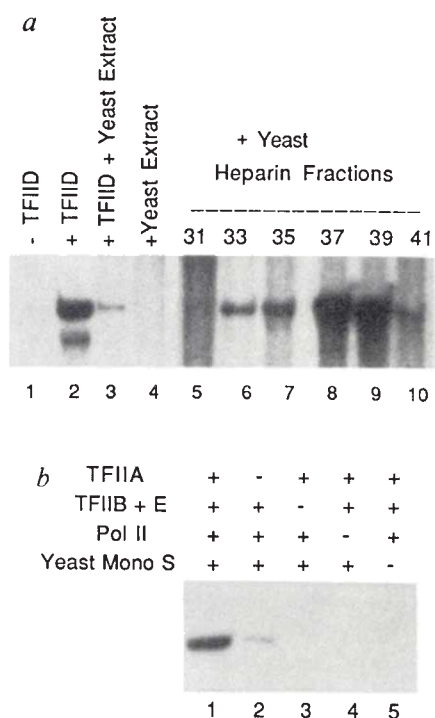


Fig. 1 *a*, Yeast extract contains an activity that substitutes for TFIID in a mammalian *in vitro* transcription system. To a TFIID-lacking transcription mix, the following additions were made: no protein (lane 1), 3 μ l of HeLa fraction DB (lane 2), 3 μ l (33 μ g) of yeast extract (lane 4), 3 μ l each of DB and yeast extract (lane 3) or 3 μ l (8–14 μ g) of fractions derived by chromatography of the yeast extract over heparin-Sepharose (lanes 5–10). The TFIID-lacking transcription mix contained the following HeLa fractions (see ref. 24 for chromatography scheme): 0.5 μ l AB (TFIIA), 0.5 μ l CB (TFIIB and E), 0.2 μ l calf thymus RNA pol II, and 1.0 μ l CD (a suppressor of nonspecific transcription initiation at nicks in the DNA). All transcription reactions contained 10 μ g ml⁻¹ of supercoiled template DNA (pML(C₂AT)₁₉Δ-51), 16 units RNasin (Promega Biotech), 60 μ M UTP and ATP (Boehringer Mannheim), 10 μ M 3' *O*-methyl GTP (Pharmacia), 10 μ M [α -³²P]-CTP (50 Ci mmol⁻¹), 60 mM KCl, 5 mM MgCl₂, 12 mM HEPES-NaOH pH 7.9, 1 mM EDTA, 0.6 mM DTT (dithiothreitol) and 12% glycerol. Total reaction volume was 20 μ l. Reactions were incubated for 60 min at 30 °C. Reactions were stopped, processed and electrophoresed as previously described²⁴. *b*, Dependence of the yeast/mammalian hybrid transcription system on individual transcription factors. The complete reaction (lane 1) contained the TFIID-lacking mix described above plus 1.0 μ l (0.67 μ g) of yeast Mono S fraction 14 (see Table 1 and Fig. 2). Individual transcription components were omitted as indicated by a minus sign. Reactions were constituted and processed as described above.

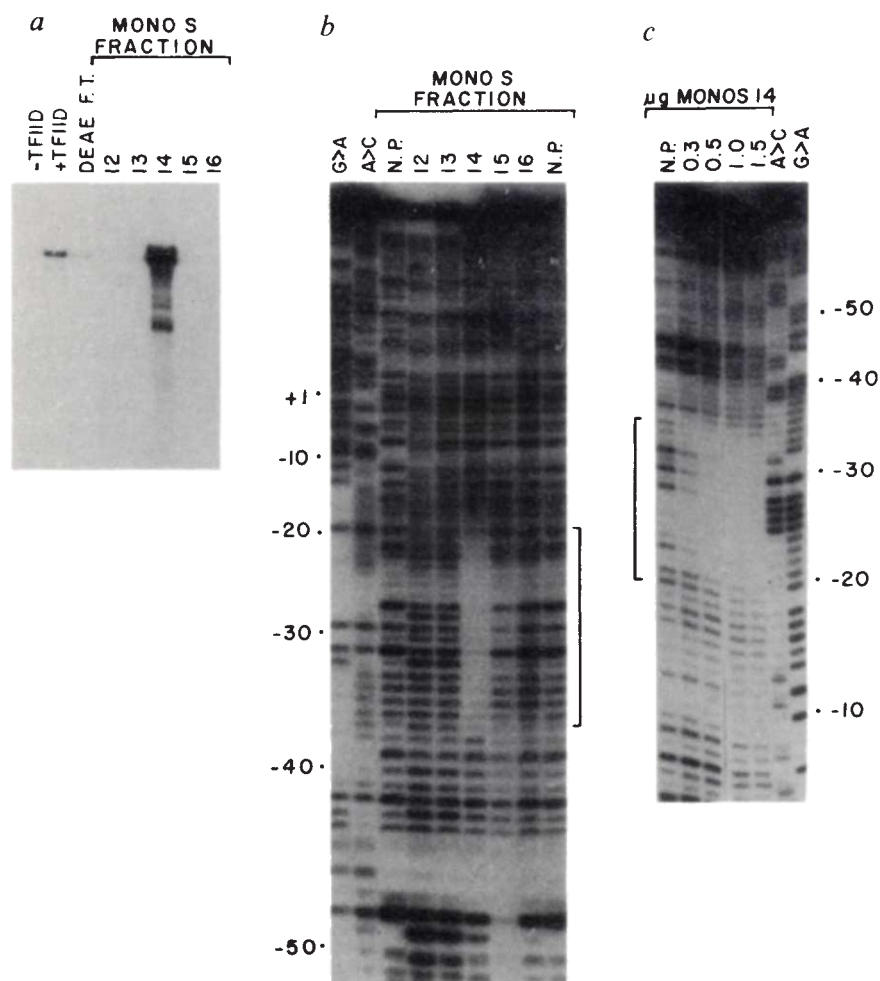


Fig. 2 Copurification of yeast TFIID-substituting activity and TATA element-binding activity. *a*, Transcriptions were constituted and processed as in Fig. 1*a*. To the TFIID-lacking transcription mix, the following additions were made: no protein ($-$ TFIID), 3 μ l of HeLa fraction DB (+TFIID), 3 μ l of the DEAE-Sepharose fraction that was loaded on to the Mono S-FPLC column (DEAE F.T.) or 3 μ l of individual Mono S-FPLC gradient elution fractions (Mono S fractions 12–16). *b*, The same Mono S fractions were tested for binding to the MLP TATA element. DNA was from plasmid pRW (ref. 35) which contained Ad2 MLP promoter sequences from -53 to $+33$ and was end-labelled on the non-coding strand. 0.5 ng of the probe fragment was incubated with 1.0 μ l of each of the Mono S-FPLC fractions for 20 min at room temperature in transcription buffer conditions. 40 ng of poly(dGdC·dGdC) was also included to absorb sequence non-specific DNA binding proteins. Reactions were then digested with DNase I, processed and electrophoresed according to a protocol adapted from ref. 37. G>A and A>C are sequence ladders of the same fragment electrophoresed as size standards. N.P. is a control reaction which did not contain yeast protein. Numbers indicate position relative to the transcription initiation site. The bracket indicates the extent of the footprint. *c*, DNase I footprint with probe labelled on the coding strand. Increasing amounts of Mono S fraction 14 were incubated with probe, digested and electrophoresed as in *b*.

merase^{28,29}. All these factors are required before formation of the first phosphodiester bond³⁰. Stimulation of transcription by regulatory proteins that bind to promoter-specific upstream sequences is mediated by enhancing the efficiency of the TATA-based reaction, because it also requires these basic transcription factors.

Given the similarity of yeast and mammalian upstream factors, it was possible that one or more of the general transcription factors might also be conserved between the two organisms. Evidence is presented here that the yeast-mammalian transcription homology extends beyond the regulatory factors and polymerase to at least one of the general transcription factors. A yeast factor that behaves like the mammalian factor TFIID in transcription and DNA binding assays is described.

***In vitro* transcription**

Accurate initiation of transcription can be reconstituted from a mammalian extract by fractions containing four factors (TFIIA, B, D and E) and purified pol II. Fractions derived from a yeast whole-cell extract⁸ were tested for the ability to complement a reconstituted mammalian transcription reaction²⁴ lacking the TATA element-binding factor, TFIID. The transcription template was the adenovirus 2 major late promoter (Ad2 MLP) from position -53 to $+10$. This 63-bp adenovirus fragment is a minimal promoter containing sequences for only the TATA box and the initiation site. This minimal promoter was fused at $+10$ to a stretch of DNA lacking Gs on the coding strand (pML(C₂AT)₁₉ Δ -51, a gift of M. Sawadogo and R. Roeder³¹). Omission of GTP from the reaction mix suppresses nonspecific background transcription, but not accurate initiation. In the

complete mammalian transcription reaction, this template directed synthesis of the expected 390 nucleotide specific transcript (Fig. 1*a*, lane 2). Addition of yeast whole cell extract to the reaction lacking TFIID did not produce a transcript (Fig. 1*a*, lane 4). Adding this yeast extract to a complete mammalian reaction (lane 3), however, severely reduced transcription (compare lanes 2 and 3), suggesting the presence of inhibitors. The yeast extract was fractionated by chromatography over a heparin-Sepharose column in an attempt to separate inhibitors from a potential complementing activity. Such an activity was unmasked and peaked in fractions that eluted at ~ 370 mM KCl (lanes 5–10). No other complementing activity was observed in the gradient fractions. Mammalian TFIID elutes from heparin-Sepharose at approximately the same salt concentration (ref. 27; S.B., unpublished data). The yeast TFIID-substituting activity was further purified over two additional columns (see Table I). In each case a single peak of activity was observed. This transcription activity flowed through a DEAE-Sepharose column at 100 mM KCl and eluted from a Mono S-FPLC column (Pharmacia) at ~ 300 mM KCl. Assuming complete recovery from each column, a maximum purification of 2,700-fold is estimated. But recovery of activity is difficult to evaluate because inhibitors of transcription were removed (see Table 1).

The transcription process mediated by mammalian TFIID is dependent on the other factors TFIIA, B and E, as well as pol II. The yeast factor was also dependent on all the other mammalian general transcription factors for activity (Fig. 1*b*). TFIIA was not essential for transcription, but stimulated the reaction at least sixfold (as determined by densitometry). A similar TFIIA stimulation was observed with transcription supported by the

mammalian TFIID (data not shown). This partial dependence could suggest that TFIIA is a stimulatory factor and not essential for initiation or that the other fractions were contaminated with TFIIA activity^{27,31}. In either case, the yeast and mammalian factors respond similarly.

As discussed previously, initiation of transcription occurs 30 bp downstream of the TATA element in mammalian cells and 60–120 bp downstream in yeast. Reactions containing the yeast factor produced transcripts of approximately the same length as the complete mammalian reaction (Fig. 1a). To test whether reactions supported by the yeast factor initiated at exactly the same site as those supported by mammalian TFIID, the 5' ends of the transcripts were analysed by an RNase protection assay³² with a resolution of a single base pair. All transcription reactions with the yeast-mammalian hybrid system initiated at the same site as the complete mammalian system, 30 bp downstream of the TATA element. This was the case whether the template was a completely viral DNA or the MLP fused to the G-lacking cassette. Both supercoiled and linear templates also showed the same start sites (data not shown).

Binding to TATA element

The specific sequence recognized by mammalian TFIID is the TATA element³³. To test whether the yeast factor which complemented the TFIID-depleted transcription system also bound to the MLP TATA element, gradient fractions from the Mono S-FPLC column were assayed in parallel for transcription and footprinting activities (Figs 2a and b, respectively). The yeast TFIID-like transcription activity peaked sharply in Mono S fraction 14. An activity which protected a region of MLP DNA from DNase I cleavage also peaked sharply in fraction 14. The protected sequences extended from -37 to -19 on the transcribed strand. The footprinting activity in fraction 14 was titrated with the probe labelled on the opposite strand (Fig. 2c). Protection was seen from -35 to -19 and saturated at 0.5 µg of protein. The footprint on both strands is approximately centred over the TATAAAA sequence (-31 to -25).

The specificity of the TATA element binding was tested by performing footprinting reactions in the presence of different competitors (Fig. 3). The competition between the probe and a

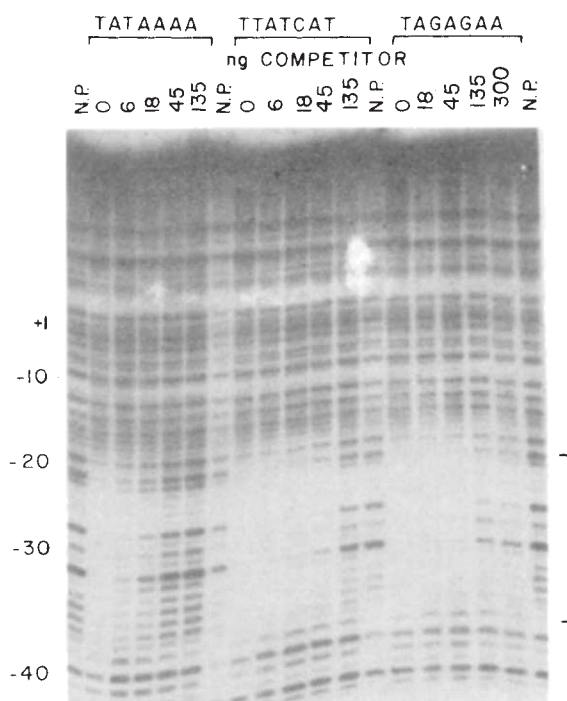


Fig. 3 Specific binding of yeast factor to a mammalian TATA element. DNase I footprint was competed by MLP fragments containing wild-type or mutant TATA elements. Reactions were done as in Fig. 2b, except for the presence of the indicated amounts of competitor DNA fragments. Competitors were synthesized chemically and cloned into the *Xma*I site of pSP65 (Promega Biotech). For footprint competitions, the competitor sequence and polylinker were excised with *Eco*RI and *Hind*III. The wild-type competitor contained MLP sequences -45 to -15 (TATAAAA). The two mutant competitors were identical except for the TATA-box mutations TATAAAA→TTATCAT(TTATCAT) or TATAAAA→TAGAGAA(TAGAGAA).

fragment containing a wild-type MLP TATA box (-45 to -15) for binding of the yeast factor was complete at 45 ng, at which point the footprint disappeared. The corresponding value for a mutant identical to the wild-type fragment except for changes in the TATA element (TATAAAA→TTATCAT) was 135 ng, whereas that for a second mutant (TATAAAA→TAGAGAA) was greater than 300 ng. Both mutants gave severely reduced levels of *in vitro* transcription with mammalian TFIID or the yeast factor (data not shown). Thus, the yeast factor specifically recognizes sequences within the TATA element that are also important for transcription.

Another property of mammalian TFIID is its participation in the formation of the first intermediate complex in the initiation reaction: a stable preinitiation complex. Formation of this complex renders subsequent transcription resistant to an otherwise inhibitory level of either poly(dIdC)²⁴, Sarkosyl³⁴ or heparin²⁷. This step is correlated with binding of TFIID to the TATA box and is stimulated by the activity of TFIIA^{26,27}. To test whether the yeast factor could form a similar stable complex, various combinations of fractions were preincubated with the transcription template before addition of inhibitory levels of poly(dIdC) and the remaining reaction components (Fig. 4a). Preincubation of the yeast factor with the template was necessary and sufficient to render transcription poly(dIdC) resistant (lanes 1 and 2). The same amount of poly(dIdC) added before the yeast factor, blocked complex formation and subsequent transcription (lanes 3–5). Formation of this preinitiation complex did not appear to require TFIIA (compare lanes 1 and 2). But, at lower concentrations of the yeast factor, addition of TFIIA to the preincubation significantly increased transcription (data not shown).

Table 1 Purification of a yeast TFIID-like protein

Purification step	Total protein (mg)	Total units transcription activity	Specific activity (units mg ⁻¹)
Crude extract	3500	0	0
Heparin Sepharose	57	1,200,000	21,000
DEAE-Sepharose	7.4	830,000	110,000
Mono S-FPLC	1.3	1,300,000	1,000,000

Samples of 138 g of *Saccharomyces cerevisiae* were grown in YPD media³⁶ and disrupted⁸ to yield a crude yeast extract. This material was chromatographed over heparin-Sepharose as previously described⁸. (In fact, the initial stages of this study were done with fractions from the heparin-Sepharose chromatography described in ref. 8.) Fractions with TFIID-like transcription activity (eluting at ~370 mM KCl) were dialysed into Buffer T + 100 mM KCl⁸ and loaded onto a DEAE-Sepharose (Pharmacia) column. Transcription activity was found in the flow-through fraction, which was loaded onto a Mono-S-FPLC column (Pharmacia). The Mono S column was eluted with a 100–600-mM KCl gradient in buffer H with 10% glycerol (identical to Buffer T⁸ but with 30 mM HEPES-KOH, pH 8.0 instead of Tris). Transcription activity peaked at ~300 mM KCl. Transcription was quantitated by adding various amounts of the yeast fractions to the TFIID-lacking reaction (described in Fig. 1 legend). Reactions were analysed by gel electrophoresis, autoradiographs were made by exposure to preflashed Kodak XAR film at -70 °C overnight, and scanned by laser densitometer (LKB). Units are arbitrary and are based on normalization to a standard reaction with mammalian TFIID.

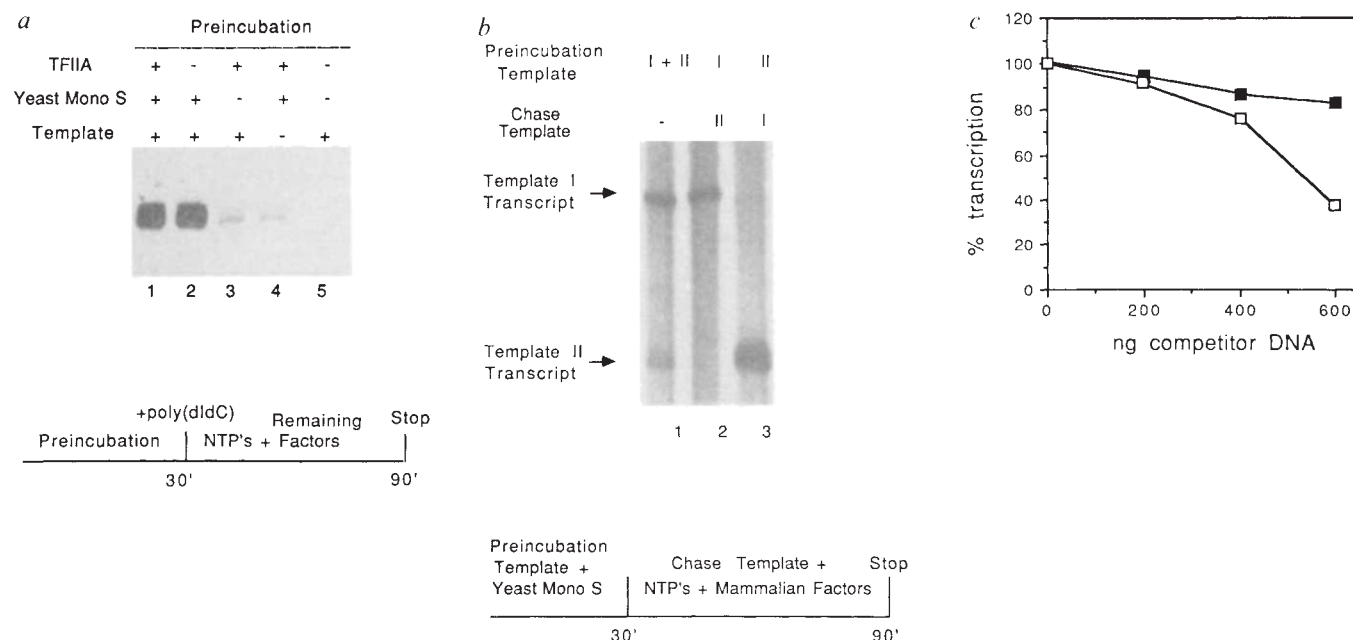


Fig. 4 Preinitiation complex formation by a yeast factor. *a*, The yeast factor is necessary and sufficient to form a complex resistant to poly(dIdC). Preincubation reactions contained 3.0 μ l (2 μ g) of yeast Mono S fraction 14, 0.5 μ l of HeLa fraction AB (TFIIA) and 200 ng of transcription template in the combinations indicated. Preincubation was in a 10- μ l reaction volume at transcription temperature and buffer conditions. After 30 min, 200 ng of poly(dIdC) was added along with pol II, nucleotide triphosphates and other transcription factors necessary to complete the reaction as described in Fig. 1*a*. The level of poly(dIdC) used inhibits mammalian preinitiation complex formation, but does not inhibit transcription after complex formation (ref. 26 and data not shown). Transcription was allowed to proceed for 60 min and processed as described earlier. Because the poly(dIdC) was added at the same time as the factors in the chase, the residual transcription in lanes 3 and 4 is probably due to a small amount of complex formed before the mix reached equilibrium. *b*, Preferential transcription of a template preincubated with the yeast factor. Preincubation contained 1.0 μ l (0.67 μ g) of Mono S fraction 14 and 200 ng of each of the indicated template DNAs (preincubation template) in 10- μ l volume at transcription buffer conditions. After 30 min at 30 $^{\circ}$ C, 200 ng of a second plasmid was added (chase template) along with pol II, the other transcription factors, and nucleotide triphosphates to complete the reaction as described in Fig. 1*a*. Transcription was allowed to proceed for 60 min and the products were processed as in Fig. 1. Template I is the standard transcription template, pML(C₂AT)₁₉ Δ -51, which gives a 390-nucleotide transcript. Template II is derived from template I by deletion of $\sim$ 180 bp from the 3' end of the G-lacking cassette, resulting in a transcript of 210 nucleotides. In control experiments, addition of both templates after preincubation of the yeast factor alone resulted in efficient transcription of both templates (data not shown). *c*, Transcription competition by plasmids containing a wild-type or mutant TATA box. 1.0 μ l of yeast Mono S fraction 14 was preincubated for 30 min at 30 $^{\circ}$ C with 200 ng of transcription template and the indicated amounts of competitor plasmids (ng competitor) at transcription buffer conditions. The other transcription components were then added, along with 200 ng of poly(dIdC) to block any transfer of bound factor, and transcription was allowed to proceed for 60 min. Reactions were processed and electrophoresed as in Fig. 1. Transcription was quantitated by scanning laser densitometry (LKB) of a linearly responsive autoradiograph and plotted as the percentage of transcription in the absence of competitor (% transcription). The wild-type competitor plasmid (open boxes) was as described in Fig. 3. The mutant (closed boxes) was identical except for the TATA box mutation TATAAAA $\rightarrow$ TAGAGAA.

Another assay of the mammalian TFIID-DNA preinitiation complex is template commitment. Preincubation of the mammalian TFIID and TFIIA factors with one template generates a complex which is sufficiently stable such that the factors will not readily exchange onto a subsequently added template²⁶. A similar template commitment was observed for the yeast factor. Complexes formed by preincubation of one template with only the yeast factor were preferentially transcribed relative to a second template added later with the remaining reaction components (Fig. 4*b*). This indicates that during the preincubation the limiting yeast factor becomes associated with one template and there is no appreciable exchange of yeast factor between templates during the reaction.

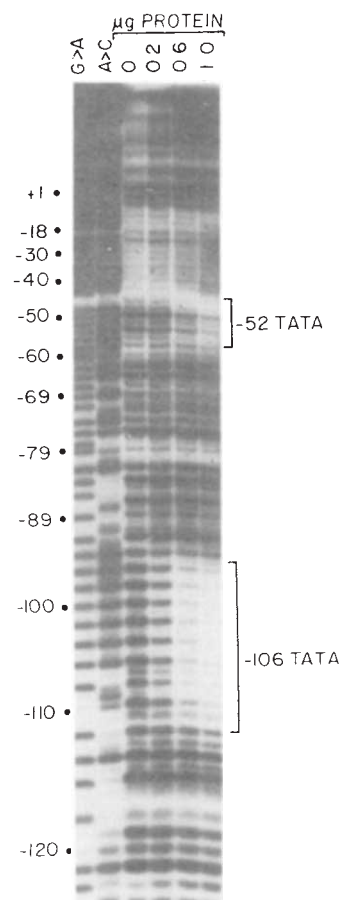
Template commitment by the yeast factor during preincubation was presumed to involve specific binding to the TATA element. To demonstrate this directly, the yeast factor was preincubated with the wild-type template and various levels of plasmid containing either a wild-type or mutant TATA box (Fig. 4*c*). The wild-type TATA element-containing plasmid competed

(TAGAGAA) plasmid. Therefore, mutations that affect yeast factor binding also affect selection of a template for transcription, supporting the hypothesis that both activities are mediated by the same protein.

Yeast transcription

Several observations further support the suggestion that the yeast factor described above is, in fact, yeast TFIID. First, preincubation of activity (Mono S column) identified by the mammalian transcription complementation assay contained activity that specifically protected two TATA elements in the yeast *CYC1* promoter (at -106 and -52) from DNase I (Fig. 5). Deletion studies have previously shown that both TATA boxes are functionally important *in vivo* for generation of a subset of *CYC1* start sites^{15,18}. The sequences of these two TATA boxes are not identical; the -52 element contains the sequence TATATAAA (closely resembling the MLP TATA box), where the -106 element contains the sequence TATATATAT. The fa

Fig. 5 The yeast factor binds to the *CYC1* TATA elements. The indicated amounts of Mono S fraction 14 were incubated with 1.0 ng of 32 P-end-labelled *CYC1* DNA from plasmid pSH Δ H3 (ref. 15). The fragment contained sequences -138 to +80 (as indicated by the numbers) relative to the most upstream initiation site of *CYC1* transcription¹⁵. Incubation and DNase I digestion were as described in Fig. 2. G>A and A>C are sequencing ladders of the same fragment electrophoresed as size standards. Footprints over the TATA elements at -52 and -106 are indicated by brackets.



either a single TATA box-binding factor with degenerate binding specificity or multiple TATA box-binding factors that are chromatographically very similar. Footprint competition assays showed that the MLP TATA element (which contains the sequence TATAAAA) competes effectively for both *CYC1* TATA footprints (data not shown), supporting the possibility that a single factor binds to both sites. A third TATA element located at -20 does not show protection at the level of yeast factor used here. The -20 element (TAAATATT) is a poor match for the consensus sequence and functions very weakly *in vivo*^{15,18}. Further experiments are required to show whether this element will bind the same factor, but with lower affinity.

A second finding suggesting that the yeast factor can recognize the *CYC1*-52 TATA element is that the hybrid *in vitro* transcription system will specifically transcribe from a template containing this sequence. Fusion of a fragment containing this element 25 bp upstream of the G-lacking cassette yielded a template that was efficiently transcribed in the mammalian or hybrid reactions. Transcription dependent on the yeast factor initiated ~30 bp downstream of the -52 TATA box, as it did with the complete mammalian transcription system (data not shown). Similar to the MLP transcription results, a system dependent on both a yeast TATA element and a yeast TATA element-binding factor initiates at a position typical of a mammalian transcription system. Therefore, it seems unlikely that the interaction of these two components determines the distance to the initiation site.

Characterization of the yeast factor

In contrast to the mammalian TFIID, the yeast factor was stable during fractionation. Although mammalian TFIID has not been purified to homogeneity, several approaches have been used to

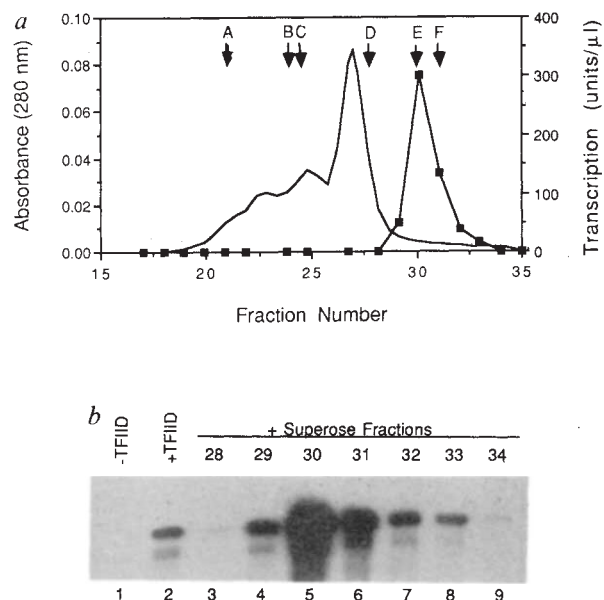


Fig. 6 Gel filtration chromatography of the yeast factor. *a*, Elution profile of the Superose 12-FPLC column (Pharmacia). 800 μ l of Mono S fraction 14 (out of 2 ml total) was concentrated to 200 μ l by centrifugation in a Centricon 10 spin concentrator (Amicon). This material was loaded on to the 25-ml column and eluted in buffer H containing 200 mM KCl and 0.1% Brij-58 (a non-ionic detergent) at a flow rate of 0.4 ml min⁻¹. 0.5-ml fractions were collected. The absorbance of the eluate at 280 nm is plotted (solid line), as is the TFIID-substituting transcription activity (black squares, see *b* for assay). Lettered arrows indicate elution peaks of sizing standards (BioRad) run on the column under identical conditions. The standards were (A) ferritin, 440K; (B) catalase, 232K; (C) bovine serum albumin, 67K; (D) ovalbumin, 43K; (E) chymotrypsinogen A, 25K; and (F) cytochrome *c*, 12.5K. The relative molecular mass of the yeast factor was estimated from a calibration plot of the elution parameter K_{av} for the protein standards (according to supplier's specifications) versus the log of the relative molecular masses and the measured elution volume of the yeast factor. *b*, Transcription activity of the Superose fractions. The following additions were made to the TFIID-lacking reaction mix (see Fig. 1): no protein (lane 1), 3 μ l of fraction DB (lane 2) or 1.0 μ l of the indicated Superose fractions (lanes 3-9). Reactions were processed and electrophoresed as previously described. Activity was quantitated as described in Table 1 and plotted in *a*. Only the peak fractions are shown; the other fractions contained no TFIID-like activity.

estimate its relative molecular mass. Glycerol gradient sedimentation showed a peak of TFIID activity at 17 S (ref. 24) and gel filtration suggested a relative molecular mass of 120-140K (ref. 27). The Mono S fraction containing the yeast factor was chromatographed on a Superose 12-FPLC sizing column and assayed for transcription activity. Surprisingly, the only fractions yielding activity suggested a relative molecular mass of 23-27K (Fig. 6). More than 50% of total activity was recovered and the chromatography resulted in an ~35-fold purification. The same Mono S fraction was resolved by glycerol gradient sedimentation analysis. The TFIID-like activity peaked at approximately 2.5 S (data not shown), consistent with the size estimate derived from the sizing column. In both analyses, footprinting of the MLP TATA element coincided with TFIID-like transcription activity.

Discussion

A probable yeast transcription factor TFIID has been isolated. The yeast factor functionally substitutes for mammalian TFIID

in a reconstituted mammalian pol II transcription system. It forms a stable preinitiation complex on the adenovirus MLP, and it binds specifically to the TATA elements of the MLP and the yeast *CYC1* promoter.

The observation that the yeast TFIID directs initiation by mammalian pol II argues for a remarkable conservation of the mechanism of transcription initiation during eukaryotic evolution. TFIID interacts not only with the promoter DNA through recognition of the TATA element, but it probably also interacts with the multi-subunit pol II complex, and perhaps with other general transcription factors such as TFIIA. Furthermore, there is evidence that regulatory proteins that bind upstream may influence transcription by interacting with the TATA element-binding factor³³. Preliminary studies have shown that the upstream activator of MLP transcription, the major late transcription factor (MLTF)^{33,35}, will specifically stimulate *in vitro* transcription from a template containing the MLTF binding site, whether the human or yeast TFIID factor is used in the reconstituted reaction (S.B., L. Chodosh, MIT; unpublished results). Thus, the yeast TATA element-binding factor can apparently interact with mammalian regulatory factors as well as mammalian initiation factors.

The mammalian and yeast TATA box-binding factors do not behave identically in all cases. The yeast factor fails to bind to a DEAE column under conditions where the mammalian factor is retained^{23,24,27}. Assuming that this does not reflect differences in proteolysis during preparation, the two proteins probably have different distributions of negative charges on their surfaces. The yeast TFIID-like factor, analysed by glycerol gradient sedimentation and gel-filtration chromatography, behaves as a protein ~23–27K. This is considerably lower than relative-molecular-mass estimates of mammalian TFIID based on similar analyses^{24,27}.

Another possible difference between the two proteins is the extent of sequences protected from DNase I cleavage upon binding to the TATA element. The yeast factor protects an 18-bp region roughly centred over the TATA element. Previous reports of the mammalian TFIID footprint on the MLP³³ and *Drosophila* TFIID footprints on several promoters²¹ are strikingly different. In these cases, the sequences protected from DNase I cleavage

extend from the same region upstream of the TATA element to encompass the initiation site 30 bp downstream. Also, nuclease hypersensitive sites that are separated by 10 bp of protected sequences are generated over a region downstream of the initiation site. The significance of the differences in these footprinting patterns is unknown. They may reflect different conformations of the same protein-DNA complex or sequential binding by multiple proteins.

One of the most interesting findings presented here is that the distance from the TATA element to the initiation site(s), which is significantly greater in yeast relative to mammalian cells, is apparently not determined by the TATA box-binding factor. Substitution of the yeast factor for mammalian TFIID does not shift the start site further downstream. Initiation is still at the characteristic mammalian initiation site 30 bp from the TATA element. Clearly, some other component of the basic transcription machinery must 'measure' this distance; perhaps one of the other general factors or the polymerase itself. One plausible model is that the proteins in the mammalian pol II complex that interact with the TFIID-TATA element complex and those that interact with the initiation site are separated by a distance of approximately three helical repeats. In the yeast pol II complex the equivalent proteins may be more flexible and further apart.

It is becoming apparent that transcription by RNA polymerase II, like other basic processes such as protein transport or splicing of RNA, is more similar than different in yeast and mammals. Indeed, in cases such as this one, the homologues from the different organisms are functionally interchangeable. Although it is not yet clear whether other components of the RNA pol II initiation process are as highly conserved as TFIID, hybrid transcription systems, combining the genetics of yeast with the biochemistry of mammalian extracts, can be a powerful tool in elucidating the mechanisms of eukaryotic transcription initiation and regulation.

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Large-scale bipolar wind in M82

Jonathan Bland & R. Brent Tully

Institute for Astronomy, 2680 Woodlawn Drive, Honolulu, Hawaii 96822, USA

In 1963 it was reported¹ that an enormous explosion ($\sim 10^{55}$ erg) had occurred about one million years ago in the nuclear region of the nearby irregular galaxy M82. Deep photographic plates showed a spectacular filamentary system extending to 200 arc s (~ 3 kpc) along the minor axis of the galaxy and H α spectroscopy revealed a steep velocity gradient in this direction, suggesting expulsion of the ionized gas from the core of the galaxy^{1,2}. Thereafter, broadband polarimetry showed the filaments to be linearly polarized (15–30%) at optical wavelengths, with electric vectors generally perpendicular to the radius vector from the centre^{3,4}, indicating that the light has been scattered by particles into our line of sight⁵. The explosion hypothesis⁶ was questionable in that the observed blueshifts and redshifts cannot arise from explosion or infall, as the particles would scatter only redshifted or blueshifted light respectively on both sides of the galaxy. Although the nature of M82 remains a mystery, two significant observations have been largely overlooked. From imaging polarimetry, Schmidt *et al.*⁷ demonstrated that most of the visual polarized flux arises from a smooth component which is symmetric over the main body of the galaxy. Axon and Taylor found well organized H α line-splitting over ~ 60 arc s at two long-slit positions south of the galaxy and parallel to the major axis⁸. Here we present new imaging Fabry–Perot observations of M82 for the H α and [N II] $\lambda 6,583$ spectral lines⁹. Our observations show for the first time that the line and continuum emission above and below the galactic disk arise from two morphologically and kinematically distinct components. The first component consists of filamentary, narrow-line emission (full width at half maximum, FWHM < 200 km s⁻¹) organized into a bipolar outflow over the surfaces of two elongated bubbles. We present direct evidence for a second component of broad-line (at FWHM 300 km s⁻¹) and continuum emission arising from a faint exponential halo that rotates slowly with the disk.

M82 was observed for 240 min on the nights of 5 and 6 February 1986 using the Hawaii tunable imaging Fabry–Perot system and a TI 512 $\times$ 512 three-phase charge-coupled device (CCD) at the CFH 3.6-m telescope. A high finesse (60), wide free-spectral-range (85 Å) QI etalon gave a velocity resolution of ~ 65 km s⁻¹ FWHM at H α . At each pixel, high-quality spectra were obtained through a 50 Å FWHM interference filter from peak intensity of the [N II] spectral line at 6,548.1 Å to peak

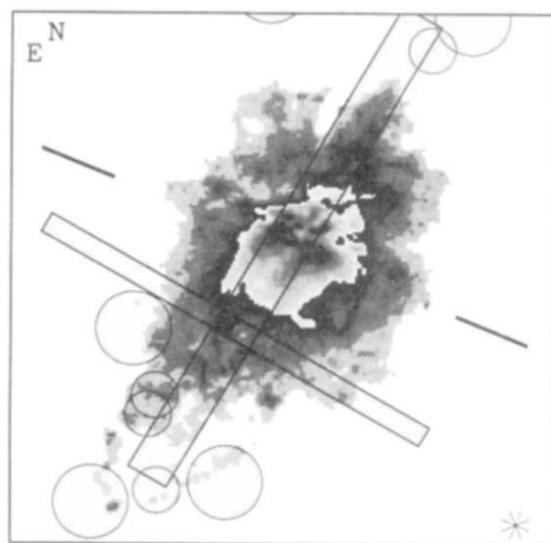


Fig. 1 An H α line flux image of M82 derived from the Fabry–Perot data. The line flux extends up to 140'' in radius along the minor axis where the major axis of the galaxy is indicated by the pointers (these are 30'' in length). The asterisk marks the position of star BD+70°587. The brightest and faintest filaments have surface brightnesses of 16 and 23 mag arc s⁻². To suppress the wide dynamic range, the intensity greyscale wraps around at a radius of ~ 25 arc s; logarithmic and linear scalings are used within and without respectively. The observed H α flux in the central region accounts for at least 70% of the H α flux over the entire galaxy. The rectangular strips indicate the spatial extent of the 'spectrograms' taken from the Fabry–Perot data presented in Figs 2 and 3. The circles show where the innermost aperture polarization measurements have been made^{17,18}.

intensity of that at 6,583.6 Å in increments of ~ 0.96 Å (44 km s⁻¹). After 2×2 on-chip binning, the square pixels subtended 0.86 arc s on the sky; the atmospheric seeing at FWHM averaged twice this value. These data comprise kinematic and photometric information for the H α and [N II] lines at $\sim 60,000$ positions over the extent of M82. Figure 1 shows an H α line flux image obtained by integrating the line profiles in the Fabry–Perot data. The procedure used to remove the continuum was biased against any broad component within the data.

Our Fabry–Perot observations confirm the H α line-splitting reported by Axon and Taylor at two long-slit positions south of the galaxy⁸. But our data show this kinematic behaviour occurs on a much larger scale. In Fig. 2*a*, we present a 'spectrogram' of the H α and [N II] $\lambda 6,583$ spectral lines extracted from the Fabry–Perot data parallel to the major axis of the galaxy. Each spectrum is binned over ten pixels perpendicular

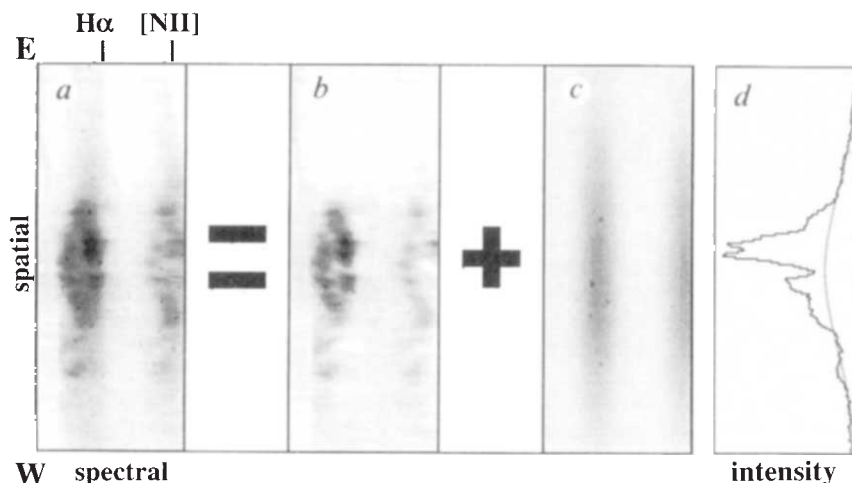


Fig. 2 *a*, A 'spectrogram' extracted from the Fabry–Perot data showing the H α and [N II] $\lambda 6,583$ spectral lines. The spectral dimension increases horizontally to the right and covers ~ 35 Å at a resolution of ~ 1.4 Å FWHM. The spatial dimension extends 172 arc s (east at top) and is roughly parallel to the major axis of M82 (see Fig. 1). The spectrogram results from the sum of two distinct components: *b*, bright filamentary narrow-line emission extending over ~ 60 arc s and *c*, a faint halo of diffuse broad-line emission. The narrow lines display a hollow ellipse structure which is especially pronounced once the broad-line halo is removed. *d*, A linear intensity tracing in the spatial direction through the brightest part of the H α line in *a*.

to the slit direction, shown by the short axis in Fig. 1. This slit covers roughly the same region discussed by Axon and Taylor. We confirm the hollow ellipse structure in both $H\alpha$ and $[N II]$ which attains a maximum separation of $\sim 300 \text{ km s}^{-1}$ close to the minor axis of the galaxy. What is not evident from the photographic plates⁸ is a diffuse component that underlies the bright filaments; its detection has required the high quantum efficiency and wide dynamic range of our linear detector.

Figure 2d is a linear-intensity tracing in the spatial direction through the brightest part of the $H\alpha$ line in Fig. 2a. The continuous line illustrates that the bright filaments are superimposed on a fainter, spatially smooth component of line emission. This component, observed everywhere within the Fabry-Perot data beyond $\sim 20''$ of the galactic disk, has some interesting properties, which makes decomposition from the bright filaments fairly straightforward. The $H\alpha$ and $[N II] \lambda 6,583$ lines have roughly constant line widths ($\sim 300 \text{ km s}^{-1}$ FWHM) which are somewhat broader than typical values for the bright filaments ($\sim 180 \text{ km s}^{-1}$ FWHM). Furthermore, the line fluxes depend only on their distance from the centre of the galaxy with $[N II]/H\alpha \approx 0.8$ in contrast to values of 0.3 to 0.5 over individual filaments. The results of the decomposition are presented in Fig. 2b and c. The observed spectrogram separates into two distinct components: bright, narrow-line filaments (FWHM $< 200 \text{ km s}^{-1}$) extending over $\sim 60 \text{ arc s}$ (b) and a faint, diffuse, broad-line halo (FWHM $\approx 300 \text{ km s}^{-1}$) spanning the length of the slit (c).

Figure 3a shows a spectrogram along the minor axis and through the nucleus of the galaxy, shown by the larger slit in Fig. 1. Each spectrum is binned over 20 pixels (17 arc s) perpendicular to the slit and shares the same spectral coverage and resolution as Fig. 2. Coverage of the red $[N II]$ line is incomplete to the north. Figure 3b and c shows that the spectrogram can again be decomposed into discrete and exponential components. Rotation of $\sim 50 \text{ km s}^{-1}$ is detected within the halo. From Fig. 3b, as the $H\alpha$ line emerges from the nucleus, it is seen to split into two parts which diverge reaching a maximum separation of $\sim 350 \text{ km s}^{-1}$ at a radius of $\sim 60 \text{ arc s}$ before rejoining at a radius of $\sim 90 \text{ arc s}$. This is seen to the south and north of M82 and confirms the generality of the behaviour exhibited in Fig. 2. The fainter branches of the split $H\alpha$ line are redshifted to the north and blueshifted to the south of the galaxy.

Hollow, elliptical line structure is observed in many galactic planetary nebulae¹⁰ and supernova remnants¹¹. That we see this behaviour perpendicular to the polar axis out to a radius of $\sim 130 \text{ arc s}$ ($\sim 2 \text{ kpc}$) argues strongly that the bright filaments reside on the surfaces of two elongated bubbles which are roughly orthogonal to the galaxy disk. The southern extension of emission observed at optical, X-ray^{12,13} and millimetre wavelengths^{14,15} is understood if the galaxy is inclined $\sim 8^\circ$, with the northern bubble tipped into the plane of the sky. In this case, the filaments define a bipolar outflow from the centre with the southern bubble moving towards us. The southern bubble is almost conical out to a radius of $\sim 65 \text{ arc s}$ where it reaches a maximum opening angle of $\sim 35^\circ$ along a position angle of $\sim 147^\circ$. The ionized gas covers the elongated bubbles as a complex network of interlinked loops and filaments. The coverage becomes more fragmentary at high galactic latitudes. The arc of luminous knots, whose apex occurs at $\sim 130 \text{ arc s}$ to the south-east, appears to define the leading surface of the bubble. The more luminous branch of the $H\alpha$ line in Fig. 3b rests near systemic velocity, which argues that one side of each bubble is almost in the plane of the sky. The fainter branch of the $H\alpha$ line zigzags through the nucleus and spans a wider range in velocity. This suggests we are seeing the opposite sides of the bubbles that subtend much larger angles to the plane of the sky. Presumably, the steep velocity gradient especially visible in Fig. 3b at $\sim 60 \text{ arc s}$ south of the nucleus is the rapid curvature of the leading surface of the expanding bubble towards the observer. This interpretation is consistent with the convergence of the split $H\alpha$ line at the apex of the bubble. By assuming the

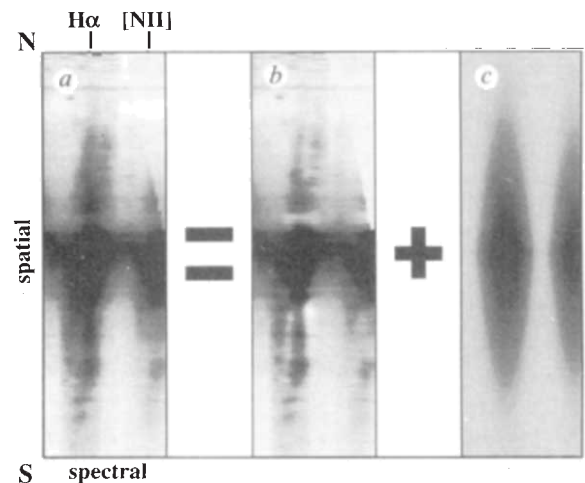


Fig. 3 a, A 'spectrogram' extracted from the Fabry-Perot data along the minor axis of the galaxy (see Fig. 1). The spatial dimension extends 215 arc s (north at top) through the nucleus of the galaxy. The spectral characteristics are the same as in Fig. 2. The spectrogram has been separated into two distinct components (b and c), as described in Fig. 2. As the $H\alpha$ line emerges from the nucleus, it branches into two parts which diverge, reaching a maximum separation of $\sim 350 \text{ km s}^{-1}$ at a radius of 60 arc s before merging again at larger radius.

outer parabolic envelope is a surface of revolution about a position angle of $\sim 150^\circ$ with one edge of the bubble in the plane of the sky, the velocity of the outermost filaments deprojects to $\sim 600 \text{ km s}^{-1}$, which implies an age of 3×10^6 years for the 2 kpc bubbles.

Over the smooth halo, the ratio $[N II]/H\alpha$ and velocity dispersion of the emission lines is constant. This argues that the light has been scattered uniformly into our line of sight. The relatively high values of the line ratio and dispersion probably originate from the obscured, circumnuclear regions as these values are not observed in the late-type disk. The scattered light provides a natural explanation for polarimetric V-band images of M82 which show a smooth, circularly symmetric halo centred on the nucleus^{7,16}. These broadband images have the same distribution as the broad-line emission observed in our Fabry-Perot data. In Fig. 1, the circles show the aperture positions of the innermost measurements where Sandage and Visvanathan have detected linear polarization^{17,18}. Most of the apertures fall outside the bright, filamentary envelope. For the small aperture enclosing the most line flux, these authors have determined the $[N II]$ and $H\alpha$ lines to be polarized at $\sim 25\%$, similar to the underlying continuum^{18,19}. Although Sandage and Visvanathan did not identify the smooth halo, a spectral decomposition for their quoted aperture size of 9.9 arc s reveals that the broad-line and continuum flux accounts for at least half the flux in their 40-Å bandpass. If the filaments are unpolarized, the intrinsic polarization from the halo may be as high as 50%. The total $H\alpha$ line flux for the halo is $5 \times 10^{38} \text{ erg s}^{-1}$ as compared with $5 \times 10^{40} \text{ erg s}^{-1}$ for the filaments. Figures 2 and 3 illustrate the need for spectropolarimetric resolution better than 3-Å FWHM to separate the two kinematic components properly.

The Fabry-Perot observations provide strong evidence that M82 is a scaled-up bipolar reflection nebula: successive supernova ejections in the starburst nucleus²⁰⁻²² have thermalized the surrounding medium which escapes along the spin axis of the thick, gaseous disk as a high-velocity, bipolar flow. Chevalier and Clegg²³ have computed the adiabatic pressure gradient in a starburst-driven wind which is qualitatively consistent with that inferred from the diagnostic $[S II]$ lines along the minor axis^{24,25}. Further evidence for a hot wind is seen in soft X-ray emission which extends over the same region as the filaments^{12,13}. For an inferred X-ray temperature of $\sim 10^7 \text{ K}$, the

hot gas is unbound for a total galaxy mass of $\sim 10^{10} M_{\odot}$ measured from our kinematic data. In the original model of Lynds and Sandage¹, the kinetic energy ($\sim 10^{55}$ erg) of the outflow is powered by an explosion at the nucleus. The inferred lifetime ($\sim 10^6$ yr) and thermal X-ray energy ($\sim 6 \times 10^{55}$ erg) enclosed by the bubbles confirms their basic idea. The bright, low-excitation filaments are likely to arise from cooling instabilities behind the moving shock surface set up by the hot wind as it ploughs into the interstellar medium. We suggest that the broad-line and continuum emission arise from reflection in a halo of dust particles that scatter light escaping preferentially along the poles from the nuclear starburst. In this model, the scattered-light component is the source of the linear polarization observed about the galaxy. As the bubbles sweep out only a fraction of the halo volume, the polarized halo is evident even along the minor axis. We expect the filaments to show essentially no linear polarization once the influence of the halo is removed. We believe the X-ray energetics and Fabry-Perot kinematics confirm finally the existence of large-scale galactic winds.

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Supernova neutrinos, neutral currents and the origin of fluorine

S. E. Woosley* & W. C. Haxton†

* Board of Studies in Astronomy and Astrophysics, Lick Observatory, University of California at Santa Cruz, Santa Cruz, California 95064, USA

† Institute for Nuclear Theory, Department of Physics, FM-15, University of Washington, Seattle, Washington 98195, USA

As the detection^{1,2} of a neutrino burst from supernova 1987A one year ago has dramatically illustrated, the flux of neutrinos generated by the collapse of the core of a massive star is truly prodigious. Common lore has it that these neutrinos, because of their weak coupling to matter, pass through all but the iron core and inner silicon shell of the collapsing star with negligible interaction. So far as energy deposition and the explosion mechanism go, this is true but for the nuclear chemistry of the star, we argue that it is not. We draw particular attention to the synthesis of an element whose origin has hitherto been obscure – fluorine – and show that its solar abundance constrains the temperature of muon and tauon neutrinos to values near what is expected from the standard model (8–10 MeV).

The idea of neutrino-induced nucleosynthesis is not new.

Domogatsky and Nadyozhin³⁻⁴ first discussed the possible production of *p*-process nuclei by charged current reaction between neutrinos from the collapsing supernova core and heavy element seed. Unfortunately, unless one can utilize *r*-process seed created from iron during the explosion itself, the flux of neutrinos at radii where elements heavier than iron will not have already been destroyed by photodisintegration will be too small to produce the *p*-process isotopes⁵. Even were *r*-process seed available, making the *p*-process in this way seems unlikely as so many neutrons would need to be removed (and not added back on). It has also been suggested⁶⁻⁷ that neutrino interactions in the hydrogen envelope might produce deuterium and other rare isotopes, especially of lithium and boron. Again, for radii sufficiently large that the fragile nucleosynthetic products would not be burned up during their ejection, the flux is too small to induce adequate transmutation. More recently, Colgate and Epstein⁸, using a cross-section estimated by one of us (W.H.), suggested an *r*-process scenario involving neutrons produced in neutral current spallation reactions (also see ref. 7). Unfortunately, the competition for free neutrons that occurs between other spallation products and the heavy seed nuclei is unfavourable to *r*-process synthesis. For example, if neutrons are produced by ${}^4\text{He}(\nu, \nu'n){}^3\text{He}$ then neutrons are also absorbed by ${}^3\text{He}$ and by protons released by ${}^3\text{He}(n, p){}^3\text{H}$. Generally speaking, neutrino nucleosynthesis has problems at two extremes. If the synthesis site is too close to the collapsing core, the fragile products of the neutrino interactions are destroyed before or during their ejection; if it is far away, the neutrino flux is small and too little synthesis occurs. Here we draw attention to the middle ground, specifically the nucleosynthesis that can occur in the carbon and neon shells of a Type II supernova.

One of us⁹ recently discussed the qualitative aspects of neutrino interactions with nuclei during supernova core collapse. Of particular interest is the inelastic neutral current scattering of a neutrino of any flavour off a nucleus in its ground (or low-lying excited) state, a process that usually transfers enough energy to produce particle emission. For the nuclear targets and neutrino energies of present interest, this process, aside from complications associated with the excitation of spin-dependent nuclear modes, can be viewed as an analogue of photoexcitation: the nuclear response is dominated by giant resonance transitions in which the nucleus is radially excited. The μ - and τ -neutrinos, because of their higher temperatures¹⁰⁻¹² compared with electron neutrinos, have greatly enhanced cross-sections for exciting these modes. We shall focus on the reactions of these neutrinos with parent nuclei that have solar¹³ (and presumably galactic) abundances greatly exceeding those of their daughter nuclei with one less proton or neutron. A particularly interesting example is ${}^{19}\text{F}$, the only stable isotope of fluorine, which has a solar abundance relative to ${}^{20}\text{Ne}$ of 1/3,100. Thus one could account for the entire observed abundance of ${}^{19}\text{F}$ by producing this isotope, in the same site where ${}^{20}\text{Ne}$ is synthesized, with a relative efficiency of only 0.03%.

According to the currently favoured models¹⁰⁻¹², the total number of μ - and τ -neutrinos and antineutrinos produced by core collapse in a massive star^{14,15} is about 5×10^{57} . The total energy in these neutrinos is approximately $(2-3) \times 10^{53}$ erg and their temperature, $\sim 8-10$ MeV, corresponds to a mean neutrino energy of 25–30 MeV. Because the process we consider is quite sensitive to the flux of neutrinos in the high-energy tail, which has a significantly higher temperature than the peak of the distribution would imply, 10 MeV is probably more nearly correct for our purposes, but we shall explore a grid of temperatures. The energy in μ - and τ -neutrinos is also about two-thirds of the binding energy of a typical neutron star, the remaining third being radiated in electron neutrinos of lower mean energy (about 15 MeV) and a correspondingly smaller cross-section for scattering off nuclei.

The cross-sections for the reaction ${}^{20}\text{Ne}(\nu, \nu'){}^{20}\text{Ne}^*$ with ν_{μ} and ν_{τ} can be estimated by folding the neutrino spectrum, which

we approximate by a Fermi-Dirac distribution, with the calculated nuclear transition strengths. The positive-parity states in ^{20}Ne were generated from the 2s1d shell model interaction of Brown and Wildenthal¹⁶. Our summation to the complete set of these states thus satisfies the Gamow-Teller sum rule. The negative-parity response was taken from the Goldhaber-Teller model¹⁷, which provides a simple description of the supermultiplet of giant resonances. The calculations are fully analogous to those described in more detail in ref. 9. The results for $5\text{ MeV} \leq T \leq 15\text{ MeV}$ are given by the approximate expression

$$\frac{1}{2}(\sigma_\nu + \sigma_{\bar{\nu}})_T = 6.5 \times 10^{-41} \left(\frac{T - 2.98\text{ MeV}}{10\text{ MeV}} \right)^{2.42} \text{ cm}^2 \quad (1)$$

yielding, for $T \approx 10\text{ MeV}$, a mean cross-section per flavour of $2.7 \times 10^{-41}\text{ cm}^2$.

These excited states of ^{20}Ne will decay principally through the neutron and proton channels, $^{20}\text{Ne}^* \rightarrow ^{19}\text{F} + \text{p}$ or $^{20}\text{Ne}^* \rightarrow ^{19}\text{Ne} + \text{n}$, with the ^{19}Ne then undergoing β -decay to ^{19}F with a half-life of 17 s. Although both reactions could in principle produce ^{19}F , the ^{19}Ne is almost entirely destroyed by other reactions, as we discuss below. Thus the branching ratio between neutron and proton emission is important. The neutron and proton yields following neutrino excitation were estimated in an optical model calculation, using a statistical level density¹⁸ in the daughter nucleus. Because the states populated in inelastic neutrino scattering are overwhelmingly isovector, competition from alpha emission was ignored: the branching ratio for isospin-forbidden alpha emission following electro-excitation in ^{20}Ne is a few per cent (ref. 19; K. Snover, personal communication). Our calculated yields for producing ^{19}F and one proton or neutron are 0.66 and 0.30, respectively, for a neutrino spectrum with $T \approx 10\text{ MeV}$. The corresponding yields from charged-current electron neutrino reactions are about an order of magnitude smaller, assuming equal luminosities in all flavours and an electron neutrino temperature $T \approx 5\text{ MeV}$. We ignore these contributions in the present calculation.

Table 1 summarizes some relevant quantities for a grid of possible temperatures. Combining the total neutral current cross-section for ^{20}Ne with the calculated neutron and proton branching ratios, one obtains the cross-sections for $^{20}\text{Ne}(\nu, \nu'n)^{19}\text{Ne}$ and $^{20}\text{Ne}(\nu, \nu'p)^{19}\text{F}$ averaged, as in equation (1), over neutrino and antineutrino types. Entries are also given in Table 1 for similar processes on ^{16}O (ref. 9) that, owing to the large abundance of ^{16}O in the regions we shall consider, are important for providing additional neutrons and protons that could destroy ^{19}F . In most massive stars the neon-rich shell (the region where carbon has at least partly burned but neon has not) lies at a distance of $(1-3) \times 10^9\text{ cm}$ from the centre of the star^{20,21}. Normalizing to a distance of $2 \times 10^9\text{ cm}$ and to a total energy in μ - and τ -neutrinos of $3 \times 10^{53}\text{ erg}$, and assuming an average neutrino energy of $3.15 T_\nu$, gives the integrated fluences of each neutrino flavour (Φ_{tot} in Table 1). The product of this fluence with the ^{20}Ne cross-section (equation 1) and the proton and neutron branching ratios then gives the total number of mass-19 nuclei produced. The ratio of these nuclei to ^{20}Ne is initially about 0.3% for $T_\nu = 10\text{ MeV}$, or an order of magnitude greater than the solar ratio of $^{19}\text{F}/^{20}\text{Ne}$. At this point, however, the survival of the ^{19}F is not yet assured. One must consider both the nuclear processing caused by the neutrons and protons liberated with the ^{19}Ne and ^{19}F (and by similar reactions on ^{16}O) and the survival of these nuclei when the shock wave necessary for their ejection passes through.

The first is an interesting problem in nucleosynthesis theory that can be readily modelled using a nuclear reaction network. A 120-isotope nuclear reaction network was constructed containing all species of interest from carbon through calcium²². These nuclei were coupled by all possible reactions involving a neutron, proton, α -particle, or photon in entrance or exit channel. A starting composition typical of the products of hydrostatic

Table 1 Cross-sections, fluences and nucleosynthesis

$T_\nu(\text{MeV})$	4	6	8	10	12
$^{16}\text{O}(\nu, \nu'n)^{15}\text{O}^\dagger$	0.0225	0.323	1.53	4.40	9.50
$^{16}\text{O}(\nu, \nu'p)^{15}\text{N}^\dagger$	0.129	1.33	5.50	14.5	29.6
$^{20}\text{Ne}(\nu, \nu'n)^{19}\text{Ne}^\dagger$	0.0932	0.933	3.55	8.61	16.1
$^{20}\text{Ne}(\nu, \nu'p)^{19}\text{F}^\dagger$	0.247	2.17	7.85	18.6	34.4
$\Phi_{\text{tot}}(\text{cm}^{-2})^\ddagger$	2.96	1.97	1.48	1.18	0.99
$X_i(^{15}\text{O}) \times 10^4$	0.042	0.42	1.5	3.3	5.9
$X_i(^{15}\text{N}) \times 10^4$	0.24	1.7	5.5	11	19
$X_i(^{19}\text{Ne}) \times 10^4$	0.084	0.59	1.7	3.1	4.8
$X_i(^{19}\text{F}) \times 10^4$	0.22	1.4	3.8	6.7	10
$X_n \times 10^5$	0.0722	0.59	1.89	3.83	6.46
$X_p \times 10^5$	0.276	1.87	5.67	10.9	17.9
$X_i(^{19}\text{F}) \times 10^4$	0.27	1.2	2.2	2.1	2.1
$[^{19}\text{F}/^{20}\text{Ne}]_i$	0.28	1.2	2.3	2.2	2.2
$[^{19}\text{F}/^{20}\text{Ne}]_f$	0.14	0.6	1.2	1.1	1.1

† Cross-section in 10^{-42} cm^2 (average for ν and $\bar{\nu}$).

‡ Total fluence (10^{38} cm^{-2}) of μ and τ neutrinos and antineutrinos at $2 \times 10^9\text{ cm}$.

carbon burning²³ was adopted: by mass 63% ^{16}O ; 30% ^{20}Ne ; 0.1% ^{21}Ne ; 0.05% ^{22}Ne ; 1.4% ^{23}Na ; 3.5% ^{24}Mg ; 0.8% ^{25}Mg ; 0.4% ^{26}Mg ; 0.6% ^{27}Al ; 0.2% ^{28}Si and traces of heavier elements at less than 0.1%. The abundances of ^{16}O and ^{20}Ne are dominant and determine the mass fractions of ^{15}O , ^{15}N , ^{19}Ne , ^{19}F , neutrons and protons (Table 1) produced as the neutrino burst passes through the neon layer. These mass fractions were added to the starting composition. This composition, at a nominal density of 10^5 g cm^{-3} , was then allowed to evolve at a temperature of 10^9 K until all neutrons, protons and α -particles disappeared. This took a short time compared to the estimated 5–10 s before the shock wave would arrive in the neon shell. Reasonable variations of temperature and density about the assumed values were found to give the same results.

As expected, a substantial fraction of the ^{19}F and ^{19}Ne was destroyed by interactions with neutrons and protons that accompanied the breakup of ^{20}Ne and ^{16}O . During the first 10^{-8} s , all the neutrons were absorbed, the chief reactions being $^{15}\text{O}(\text{n}, \text{p})^{15}\text{N}$, $^{19}\text{Ne}(\text{n}, \alpha)^{16}\text{O}$, $^{20}\text{Ne}(\text{n}, \gamma)^{21}\text{Ne}$, $^{19}\text{Ne}(\text{n}, \text{p})^{19}\text{F}$, $^{15}\text{N}(\text{n}, \alpha)^{12}\text{C}$, and $^{24}\text{Mg}(\text{n}, \gamma)^{25}\text{Mg}$. By this point the ^{19}Ne abundance was reduced by about a factor of three compared to its starting value and ^{19}F had increased slightly. During the next microsecond, protons were consumed by $^{15}\text{N}(\text{p}, \alpha)^{12}\text{C}$ and $^{19}\text{F}(\text{p}, \alpha)^{16}\text{O}$. A substantial fraction of the protons was also consumed by $^{23}\text{Na}(\text{p}, \alpha)^{20}\text{Ne}$. Alpha-particles produced by these reactions mostly added to ^{16}O and ^{20}Ne , causing no major readjustment in important abundances. In the end, when the neutron, proton, and α -particle abundances had all declined below 10^{-15} , the abundance of ^{19}F was as given in Table 1 ($X_f(^{19}\text{F})$).

We expect that no appreciable error was introduced into the calculation by assuming the instantaneous production of all ^{19}Ne and ^{19}F . In reality these species would be continually produced over a period of about ten seconds, but as the abundances of all of the other isotopes competing effectively for neutrons and protons either did not change appreciably during the calculation (for example, ^{23}Na) or were produced at a rate proportional to that of n , p , ^{19}Ne , and ^{19}F (^{15}O , for example), the final results should be insensitive to this assumption. To test this statement the calculation at 8 MeV was repeated assuming the same total neutrino flux, but a time dependence $\phi(t) = 4.93 \times 10^{37} \exp(-t/3\text{ s})$. Neutrino capture was incorporated into the network in analogy to the photodisintegration reactions, (γ, n) and (γ, p) . The initial composition was as before, apart from the absence of ^{15}N , ^{15}O , ^{19}Ne , ^{19}F , neutrons or protons. Although no large neutron or proton abundances were generated, the net result, when the neutrino burst had subsided, was

a ^{19}F mass fraction of 2.7×10^{-4} , a value quite similar to that in Table 1.

Also in Table 1 is the quantity $[^{19}\text{F}/^{20}\text{Ne}]$ defined as the ratio of the 'final' fluorine to neon mass fractions compared to their ratio in the Sun. A value of one would indicate exact synthesis relative to neon, but because more fluorine will be destroyed during ejection than neon (see below) a value larger than one is necessary. An important implication is that the temperature of the μ - and τ -neutrinos in the neon- and fluorine-producing events had to be greater than 4 MeV and probably greater than about 6 MeV. The fact that $[^{19}\text{F}/^{20}\text{Ne}]$ approaches, at high temperature, a constant value of ~ 2 is a consequence of the competition with ^{23}Na for protons. So long as the abundance of ^{19}F remains large, all of the available protons destroy ^{19}F by $^{19}\text{F}(p, \alpha)^{16}\text{O}$. But once the ^{19}F abundance has declined below a critical mass fraction, $\sim 2 \times 10^{-4}$, the remaining protons go on sodium. This has the interesting implication that over the course of galactic history the abundance of fluorine will have tracked that of sodium more than that of neon. Fluorine, like sodium, is then technically a 'secondary' element. In Population II stars, formed in the distant past with a much smaller initial abundance of carbon, nitrogen, and oxygen than massive stars forming today, ^{19}F production will be reduced.

The second factor one must consider in calculating fluorine nucleosynthesis is its possible destruction as the shock wave responsible for exploding the star propagates through the neon shell. Passage of the shock gives rise to an 'explosion temperature'²⁴ approximately equal to $(3E_0/4\pi r^3 a)^{0.25}$, with a the radiation constant. For a radius of $(1-3) \times 10^9$ cm, this implies peak temperatures $\approx (1.0-2.5) \times 10^9$ K. More of the mass is situated at larger radii and experiences the lower shock temperatures (see Fig. 5 of ref. 24). These temperatures will decline exponentially with a time constant $\approx 446/\rho^{0.5}$ s (ρ = density), or ~ 1 s. The same reaction network used before to study neutron and proton interactions shows that the chief reaction responsible for fluorine destruction at such high temperatures is $^{19}\text{F}(\gamma, \alpha)^{15}\text{N}$. For temperatures $< 1.7 \times 10^9$ K the fluorine will survive shock wave passage. At higher temperatures (smaller radii) it is destroyed. Although, as stated above, more mass experiences the lower temperatures, the neutrino flux responsible for the synthesis of fluorine declines as r^{-2} , so that less fluorine is produced beyond the mean Ne shell radius of 2×10^9 cm. However, as Table 1 shows, if less fluorine is made, other species, specifically ^{20}Ne and ^{23}Na , compete more effectively for the neutrons and protons that can destroy ^{19}F . Coupling all of this together is a complicated problem for the future, but we estimate that roughly one-half of the fluorine produced (after the n, p, and α -particle abundances have gone to zero) will survive ejection. Dividing $[^{19}\text{F}/^{20}\text{Ne}]$ by two in Table 1 gives $[^{19}\text{F}/^{20}\text{Ne}]_f$, the final ejected abundance ratio of fluorine to neon compared to the Sun.

Putting both destruction mechanisms together, $^{19}\text{F}(p, \alpha)^{16}\text{O}$ before shock passage and $^{19}\text{F}(\gamma, \alpha)^{15}\text{N}$ after, Table 1 suggests that, for neutrino temperatures near the currently accepted values (8–10 MeV), fluorine will be produced in adequate proportions relative to neon. Although one should bear in mind a typical uncertainty of a factor of two in the cosmic abundances of neon and fluorine, if supernovae Type II are the production site of ^{20}Ne , as most researchers believe²⁴, then the process described above could account for the fluorine in our galaxy (and in our toothpaste). This is gratifying because no satisfactory nucleosynthesis mechanism for fluorine has existed previously.

We also note in closing that other rare isotopes also might be produced in this manner. The reactions $^{181}\text{Ta}(\nu, \nu'n)^{180}\text{Ta}$ and $^{139}\text{La}(\nu, \nu'n)^{138}\text{La}$, in particular might be responsible for the synthesis of these rare daughter isotopes. In the Sun $^{181}\text{Ta}/^{180}\text{Ta} = 8,100$ and $^{139}\text{La}/^{138}\text{La} = 1,100$. These species will be produced with greater efficiency than ^{19}F because they will not be destroyed appreciably by neutrons (the proton channel is negligible) and they will survive photodestruction up to a

temperature of nearly 2×10^9 K. On the other hand, as the abundance of neon in a massive star will far exceed that of these other parent nuclei, establishing supernovae as the principal site for the nucleosynthesis of ^{180}Ta and ^{138}La may prove more difficult. Finally we note the production, along with ^{19}F in the neon shell, of interesting quantities of radioactive ^{26}Al . Typically ^{26}Al is produced by $^{25}\text{Mg}(p, \gamma)^{26}\text{Al}$ at a level about 0.2% that of ^{27}Al , a value known to be significant to γ -line astronomy of the interstellar medium^{25,26}. Finally we point out an interesting mode of lithium and boron synthesis that may go on in the carbon shell (see also ref. 7). There $^{10,11}\text{B}$ can be produced by $^{12}\text{C}(\nu, p)^{11}\text{B}$, $^{12}\text{C}(\nu, n)^{11}\text{C}(e^+ \nu)^{11}\text{B}$, and $^{12}\text{C}(\nu, np)^{10}\text{B}$. Lithium is then produced by the important secondary reaction $^{10}\text{B}(p, \alpha)^7\text{Be}$ which decays after ejection to ^7Li . We expect that other interesting examples of this nucleosynthesis process can also be found.

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Highly fractionated rare-earth elements in ferromagnesian chondrules from the Felix (CO3) meteorite

Keiji Misawa*† & Noboru Nakamura*†

* Department of Science of Material Differentiation, Graduate School of Science and Technology, and

† Department of Earth Sciences, Faculty of Science, Kobe University, Nada, Kobe 657, Japan

Chondrules and Ca, Al-rich inclusions (CAIs) are important constituents of chondritic meteorites which preserve a record of chemical fractionation at high temperature in the early solar nebula¹. Cosmochemical research on these components of meteorites shows that chondrules were formed by melting of pre-existing solid precursor materials^{2–4}. Although highly fractionated rare-earth element (REE) patterns have been known for CAIs^{5,6} and are considered as evidence for high-temperature fractionation

† Present address: Institute for Cosmic Ray Research, University of Tokyo, Tanashi, Tokyo 188, Japan.

in the nebula^{7,8}, little is known about refractory precursor components of chondrules. In addition, chondrules so far studied rarely exhibit fractionated REE patterns, except in a few cases^{9,10}, so that no single variety of refractory precursor component can be invoked as the fractionated REE carrier in chondrules. Here we describe two ferromagnesian chondrules from the Felix (Ornans-subtype) carbonaceous chondrite which carry a marker signature of REE fractionation in the nebula. Both show positive Ce and Yb anomalies and one exhibits a light/heavy REE fractionation similar to those of Group II CAI⁶. On the basis of the REE characteristics of these chondrules, as well as those of Allende (CV) (ref. 11), we suggest that one of the precursor materials of chondrules in CO-CV carbonaceous chondrites is a high-temperature condensate from the nebular gas.

Chondrules were separated mechanically from a sample of Felix (USNM 235) by freeze-thaw processing. After ultrasonic cleaning in distilled acetone each chondrule was broken into two parts using an agate mortar. One half was used to prepare a thin section, and the other half was used for trace element analyses. Petrographic examination was performed using a scanning electron microscope (SEM) equipped with an energy dispersive spectrometer (EDS) and an electron probe microanalyser (EPMA). They were operated at an accelerating voltage of 15–20 kV and a beam current of 1.5–12 nA.

The chemical composition of constituent minerals (olivine and pyroxene) of chondrules numbered 3 and 8 were determined by EPMA focused beam analyses. Si, Al-rich mesostasis of chondrule 8 was analysed by the SEM-EDS area scan. The bulk chemical compositions of silicate portions (excluding opaque minerals) of chondrules were determined by EPMA defocused beam analysis for chondrule 3, or by combining EPMA and SEM-EDS analyses of constituent phases with modal analyses for chondrule 8. Abundances of REE, K, Rb, Sr, Ba and Ca were determined precisely by the mass spectrometric isotope dilution (MSID) technique¹². Results are shown in Tables 1 and 2. Chemical compositions of McSween's type III^{13,14} and Wark's CA (Ca-Al-rich) chondrules¹⁵ are also presented in Table 1.

Chondrules 3 and 8 exhibit porphyritic olivine-pyroxene and

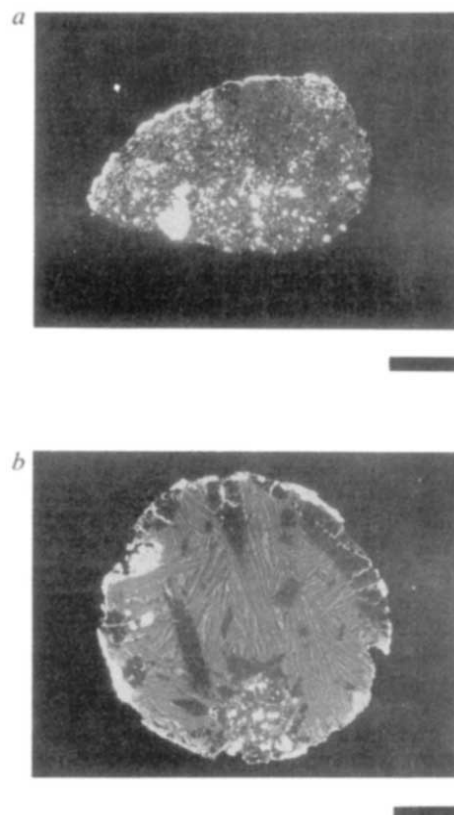


Fig. 1 Back-scattered electron images of chondrules from the Felix (CO3) carbonaceous chondrite. *a*, Chondrule 3 is microporphyritic texture with low- and high-Ca pyroxene ($Wo_{1.4-6}$, $En_{98-88}Fs_{0.5-5.5}$, $Wo_{33}En_{67}$), olivine (Fa_{3-7}), abundant metallic Fe, Ni and Fe-sulphide. *b*, Chondrule 8 contains a small low-Fe olivine (Fa_{4-9}) aggregate (lower bottom). Enstatite (containing Al_2O_3 ~3 wt%) phenocrysts (up to 400 μm in long dimension) are embedded in a fine-grained dendritic Si, Al-rich mesostasis. Iron-sulphide forms discrete rims around both chondrules. Scale bars in *a* and *b* are 200 μm .

Table 1 Per cent bulk composition of silicate portions of two chondrules from the Felix (CO3) meteorite as determined by SEM-EDS* and EPMA†

	Felix chondrules		Average of type III (ref. 14)	Range of CA chondrules (ref. 15)
	3‡	8§		
SiO ₂	45.0	52.5	52.4 (4.1)	40–53
TiO ₂	n.d.	0.3	0.28 (0.17)	0.2–0.8
Al ₂ O ₃	7.5	13.8	7.65 (4.93)	11–23
Cr ₂ O ₃	0.6	0.8	0.54 (0.24)	
FeO	7.5	2.8	7.45 (3.77)	
MnO	0.1	0.3	0.24 (0.17)	
MgO	33.1	18.9	23.0 (9.1)	9–22
CaO	6.0	6.5	7.26 (3.80)	7–16
Na ₂ O	0.3	3.8	1.81 (1.50)	
K ₂ O	n.d.	0.2	0.80 (0.11)	

EPMA and SEM-EDS data were corrected by standard procedures (ref. 27) or ZAF method respectively. Data obtained by EPMA defocused beam analyses were further corrected by the method of Ikeda (ref. 28). Analyses were adjusted to 100%. n.d., Not determined.

* Model Hitachi S530 SEM equipped with a Horiba EMAX-2200 EDS.

† Model JEOL JCSA-733 EPMA.

‡ EPMA defocused beam analysis (50 μm diameter, for over 700 s count) avoiding opaque minerals.

§ Determined by combining analyses of EPMA focused beam and SEM-EDS area scan for constituent phases with modal analyses, excluding opaque minerals (see text).

|| Numbers in parentheses represent one standard deviation¹⁴.

porphyritic pyroxene textures¹⁶ respectively (Fig. 1). It appears that chondrule 3 (Fig. 1a) can be assigned to a common ferromagnesian type. Chondrule 8 (Fig. 1b) is poor in Fe and richer in Al than those of type III, and transitional in composition from type III to type IV (ref. 14) or CA chondrules.

Figure 2 shows CI-chondrite normalized trace element abundance patterns for chondrules 3 and 8. Enrichment factors of moderately volatile K and Rb, and refractory Sr and Ba are $1.4\text{--}3 \times CI$ and $4\text{--}11 \times CI$, respectively. In spite of the high volatility of alkali metals at the melting temperature, these chondrules have retained the moderately volatile elements at levels higher than chondritic, suggesting that there has been no appreciable vaporization loss of more refractory elements (such as REE) in the chondrule melting event. The REE features observed in the chondrules studied should therefore not reflect the chondrule-formation melting event, but the nature of precursor materials.

Chondrule 8 exhibits the most highly fractionated REE pattern so far reported for chondrules. The pattern is similar to those of Group II CAIs^{6,17,18}, but differs from them in detail. The chondrule has a large positive Ce anomaly (50%) and shows light REE fractionation with a monotonic increase from La to Sm, but the Eu anomaly common in Group II CAIs is not observed. As a result, the REE pattern appears to be a new type of Group II, or could possibly be related to Group IIA of Davis and Grossman⁸. Chondrule 3 also shows anomalies of both Ce and Yb, but in addition has a slight positive Eu anomaly. The general REE pattern for chondrule 3 (minor light REE enrich-

Table 2 Bulk chemical compositions* obtained by the MSID technique for chondrules from the Felix (CO3) meteorite (Values in p.p.m. unless otherwise stated)

Sample dissolved	Chondrule	
	3 408 (μg)	8 365 (μg)
Ca (%)	4.72	4.21
K	1,020	1,830
Rb	3.25	7.02
Sr	74.0	88.7
Ba	9.42	13.5
La	0.688	1.70
Ce	2.37	6.76
Nd	1.30	3.76
Sm	0.457	1.74
Eu	0.210	0.199
Gd	0.514	0.414
Dy	0.587	0.379
Er	0.383	0.142
Yb	0.668	0.374
Lu	0.0608	0.0191

* Analytical precision is considered to be 1–3%.

ment and a positive anomaly at Ce) could be explained by mixing of 90–92% of 2.35–2.45 $\times$ CI-chondrite (flat REE pattern) and 8–10% of chondrule 8. Similarly, the REE pattern of chondrule 8 is interpreted as a result of mixing an even more fractionated REE component with an unfractionated REE component. Thus, a refractory lithophile precursor with extremely fractionated REE seems to have been common to both chondrules 3 and 8.

It was shown that one pyroxene-rich chondrule (number 9) in the Allende CV3 chondrite has the same kind of REE pattern¹¹, and that hibonite grains in the Murchison CM2 chondrite¹⁹ and CAIs from the Mokoia CV3 chondrite²⁰ also appear to have REE patterns similar to those of the Felix chondrules reported here. Hence, the unique REE fractionation represented by chondrule 8 may be a common feature for some type of Al(Ca)-rich chondrules and/or CAIs, and is considered to indicate some physical and chemical processes in the nebula.

Based on the correlation of the linear isotope effects for Mg and Ca, it is suggested that CAIs may have been formed by multiple stages of condensation, vaporization, and recondensation²¹. By analogy with the formation history of CAIs, refractory precursor materials of chondrules are considered to have experienced a similar high-temperature process.

Although Al-rich and/or Ca, Si-rich objects could have been produced by partial melting of primitive dust aggregates²², this process may be inconsistent with the relatively high volatile element abundances for the Felix chondrule 8 with higher Al and Si. Clearly, REE features of the chondrule cannot be due to any solid/liquid fractionation process.

It has been suggested that REE will condense as a solid solution in high temperature minerals, and that the highly fractionated REE component of Group II CAIs was established when the REE in the gas phase were in equilibrium with an ultrarefractory component^{7,8}. In addition, the results of equilibrium condensation calculations show that the anomaly of Ce is closely related to the gas/solid processes in an oxidizing environment^{23,24}. Thus, the presence of positive anomalies at Ce for Group II patterns suggests that their complementary ultrarefractory components were removed under more oxidizing conditions than the canonical solar nebula.

According to a recent condensation calculation²⁵, Ce oxides condense at a significantly lower temperature than those of La and Nd. If this is the case, and the temperature at which the ultrarefractory component was removed from the gas phase was

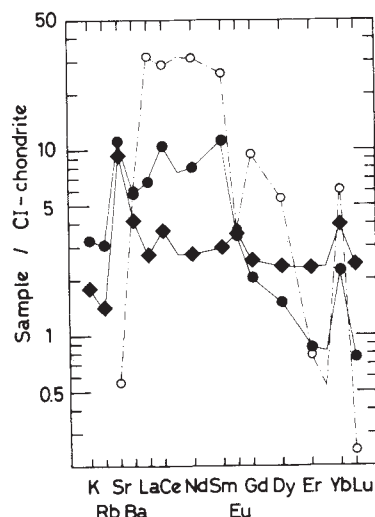


Fig. 2 CI-chondrite¹² normalized trace element abundance patterns for chondrules 3 (squares) and 8 (filled circles) from Felix (analytical errors are within the symbol size). Chondrule 8 shows the most fractionated REE pattern ever reported in chondrules. Positive anomalies of Ce and Yb are recognized in both chondrules. The general REE pattern for chondrule 3 could be explained by mixing of chondritic component and chondrule 8 (see text). Typical Group II REE pattern (Allende CAI G_p; ref. 5) is also shown (open circles).

relatively low (about 1,621 K; 50% condensation temperature of La₂O₃ at 10⁻⁴ bars total pressure²⁵), it is possible that Ce was preferentially partitioned into the gas phase relative to neighbouring REE. A total condensate of this residual gas should have a Group II REE pattern with a positive Ce anomaly.

From the above discussion, we conclude that one of the possible precursor materials of chondrules in CO-CV chondrites is a high-temperature condensate. It was formed from the residual gas after removal of an ultra-refractory component under relatively oxidizing and/or relatively lower temperature conditions in the solar nebula^{7,8}. Alternatively, they could have occurred in the pre-solar environment²⁶. This component was subsequently mixed with the precursor materials of the more common ferromagnesian chondrule in the chondrule formation process. The suggestion implies that the identity of different components formed by the chemical fractionation of the nebular gas during high-temperature condensation and/or evaporation was not destroyed by whatever thermal process formed the chondrules.

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Entropy and enthalpy catastrophe as a stability limit for crystalline material

H. J. Fecht & W. L. Johnson

Keck Laboratory of Engineering Materials, 138-78,
California Institute of Technology, Pasadena, California 91125, USA

The transition from a crystalline material with long-range order to a glass-like disordered structure has been observed in several metal alloys and minerals using experimental techniques such as solid-state reaction, mechanical alloying, pressure application, ion-beam mixing and hydriding¹⁻³. In all these examples the vitrification occurs below the glass transition temperature, and because the glass can be thought of as a highly undercooled liquid, one may draw an analogy between the vitrification process and melting. The melting process is often viewed as a catastrophic instability of the crystal lattice⁴⁻⁶, although none of these theories predicts the melting temperature correctly. The transition from crystal to glass could also be triggered by some type of instability⁷; indeed, Kauzmann⁸ has argued that an undercooled liquid whose entropy falls below that of the crystalline phase must undergo massive freezing to a glass. Applying an 'inverse' Kauzmann argument to the problem of melting, an entropy catastrophe is predicted when the entropy of a superheated crystal exceeds that of the liquid phase. We propose that this temperature represents an ultimate stability limit for superheated or supersaturated crystals.

Superheating experiments indicate that melting is usually a process nucleated at heterogeneous nucleation sites such as grain boundaries and free surfaces. The resistance to melting is very small in metals⁹, moderate in minerals^{10,11}, whereas solid He can be superheated up to 1.56 times the thermodynamic melting point T_m (ref. 12). Providing heterogeneous nucleation could be avoided for melting, the stability limit of a superheated crystal would be given by a thermochemical instability resulting in homogeneous disordering and catastrophic melting. By analogy to the ideal glass transition temperature, T_g , defined by the isentropic condition for an undercooled liquid and crystal below the T_m , the instability temperature T_i^s can be obtained by determining the isentropic temperature for a superheated crystal and a liquid above T_m . The apparent paradox implied when the entropy of a liquid phase falls below the entropy of the corresponding crystalline phase can thus be extended to the case of a superheated crystal. Measurements of the heat capacity c_p for metals indicate that c_p of the crystal and liquid become equal within 100°C of the melting point. Calculating the enthalpy and entropy changes as a function of temperature for the crystal and liquid therefore leads to a maximum enthalpy and entropy difference between crystal and liquid close to T_m . At elevated temperatures, c_p of the crystal consists of a roughly

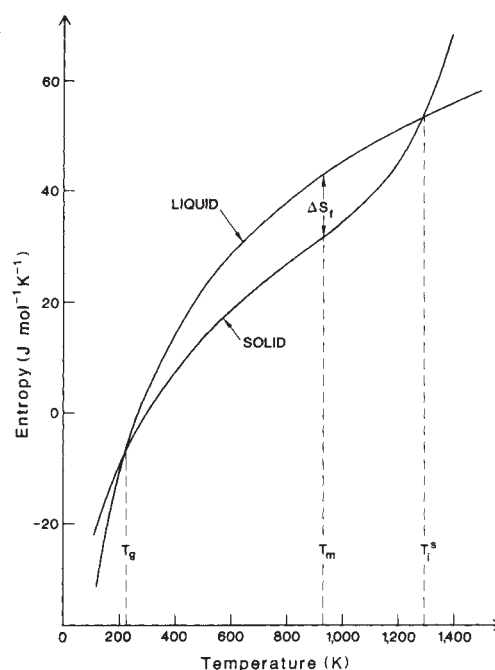


Fig. 1 The entropy of liquid and crystalline aluminium as function of temperature in the stable and metastable regime. ΔS_f denotes the entropy of fusion.

linear term, c_p^e , resulting from thermal expansion and electronic heat capacity, a contribution c_p^v due to thermal vacancy formation and a Debye-like term c_p^D which approaches the Dulong-Petit value of $3R$ at high temperatures. For aluminium, the entropies of the crystal and the liquid were calculated on the basis of available c_p data for the crystal¹³ and the stable¹³ and undercooled liquid¹⁴ phase as $S(T) = \int_{T_m}^T c_p/T dT$, and are shown in Fig. 1. An ideal glass temperature T_g , of 225 K ($0.24 T_m$) is obtained. When the vacancy contribution was neglected, a critical temperature T_i^s (where the entropy of the crystal equals that of the liquid) of $\sim 3 T_m$ is found. On the other hand, if the vacancy contribution is included, a much lower value of $T_i^s = 1.38 T_m$ is obtained as shown in Fig. 1. It is therefore seen that T_i^s is strongly dependent on the proper description of the vacancy heat capacity.

Considering the formation of vacancies as a feature of thermodynamic equilibrium of crystalline material and neglecting vacancy/vacancy interactions, c_p^v is obtained as $c_p^v = [(\Delta H^v)^2/RT^2]A \exp(-\Delta H^v/RT)$ in the dilute limit with the heat of formation of a vacancy, ΔH^v , held constant. For aluminium, ΔH^v and the constant A are obtained from heat capacity measurements and known vacancy concentrations close to T_m (ref. 15). Extrapolating these data into the superheated range using this approximation leads to an instability temperature of $T_i^s = 1.38 T_m$ for crystalline superheated Al. Figure 1 shows the entropies obtained for liquid and crystalline Al with $T_i^s = 1,292$ K. The obtained value is considered as an upper bound for the isentropic instability temperature. In reality, ΔH^v is dependent on temperature and vacancy concentration, and the vibrational entropy contribution associated with vacancies is not negligible. If the dependence of ΔH^v on the vacancy concentration $c^v(T)$ is taken into account as $\Delta H^v(T) = \Delta H^v(T_m)(1 - c^v(T))$, the instability temperature is found to be 1,234 K within this approximation. Considering the vibrational entropy term $S^{\text{vibr}} = 3k(1 + \ln T/\theta_E)$, θ_E being the Einstein temperature, would reduce T_i^s even further because θ_E decreases with increasing c^v . This entropic instability of a superheated

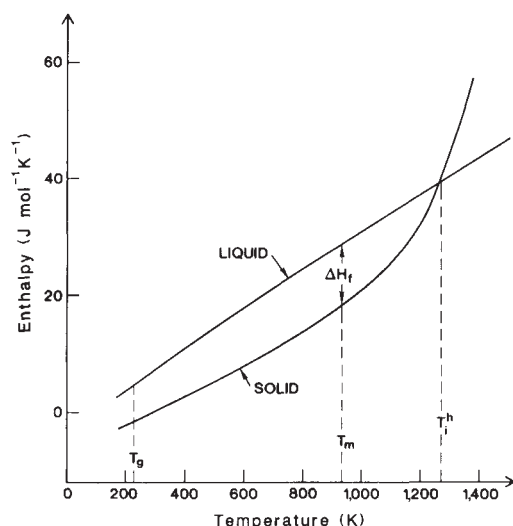


Fig. 2 The enthalpy of liquid and crystalline aluminium as function of temperature in the stable and metastable regime. ΔH_f denotes the heat of fusion.

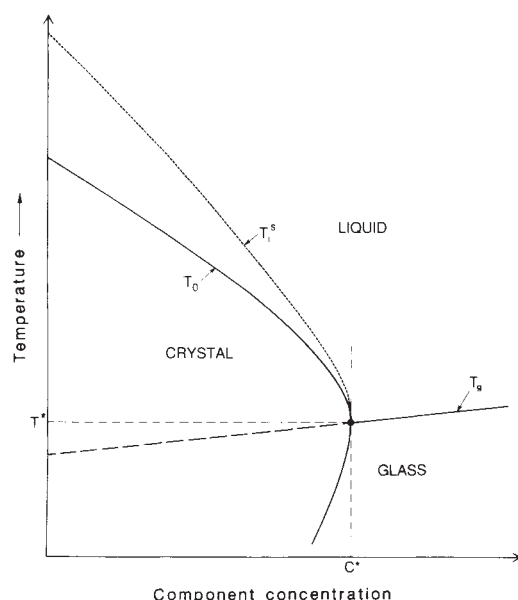


Fig. 3 Schematic metastable polymorphous phase diagram which includes the instability temperature, T_i^s , the melting temperature, T_0 , and the glass temperature, T_g , and defines the stability range of the crystal, liquid and glass phases.

Al-crystal occurs at a temperature well below that at which extrapolated elastic moduli vanish ($1.6 T_m$) (ref. 1).

In a similar way, the enthalpy changes of liquid and crystalline Al as function of temperature were calculated as $H(T) = \int_{T_m}^T c_p dT$ and are shown in Fig. 2. Assuming ΔH^v is constant, an upper bound of 1,272 K for the isenthalpic temperature T_i^h is obtained. Corrections for ΔH^v corresponding to $c^v(T)$ dependence would reduce T_i^h to a value of 1,217 K. Above the isenthalpic temperature T_i^h , melting becomes exothermic and is no longer heat-flow limited.

The vacancy concentration required to destabilize the crystalline material at T_i^s is found to be about 10%. This value relates closely to the volume change involved during melting. Hence, if the instability temperature T_i^s , where the entropy of fusion

vanishes, coincides with the temperature where the volume of the liquid and the superheated crystal become equal, melting occurs continuously and becomes a second-order phase transition. In general, this would occur at an isolated critical point. Below the glass transition-temperature T_g , a vacancy concentration of 5% (or a corresponding concentration of other defects like grain boundaries or dislocations) is required to raise the enthalpy of the crystal above the value for the amorphous phase. This defect concentration is considerably smaller than the defect concentration required for amorphization of a Lennard-Jones crystal in a molecular dynamics simulation¹⁶, but has not been reached experimentally for pure metals owing to recovery effects, therefore avoiding solid-state amorphization of pure metals. Similar results based on c_p data and vacancy concentrations have also been obtained for W (ref. 17) and Nb (ref. 18) with predicted entropy instabilities at 1.18 and 1.43 T_m respectively.

In what follows, the concept presented for the evaluation of thermochemical instability criteria of crystalline material has been extended to the case of binary alloys and the problem of solid-state amorphization. For a two-component system, the free-energy diagram has a compositional degree of freedom in addition to T and P . In most glass-forming alloy systems the resulting phase diagram is characterized by plunging T_0 lines, T_0 being the temperature of equality of the free energy of the solid solution and the liquid phase¹. The T_0 line therefore denotes the polymorphous melting curve of the crystal in the T - c plane. Consequently, a polymorphous phase diagram is constructed including glass, liquid and crystal as shown in Fig. 3, and illustrates the possible thermodynamic states of a metastable system constrained to be a single phase. The T_0 line, that is, the 'melting' temperature of the solid solution, and the isentropic temperature T_g (ideal glass temperature) cross at a certain composition c^* and temperature T^* . At this condition, the slope of the T_0 line, dT/dc , which equals $(\partial(G^l - G^s)/\partial c)/\Delta S$, becomes infinite because the entropy difference ΔS between crystal and undercooled liquid becomes zero. (The actual glass temperature, where the undercooled liquid is topologically frozen, is slightly higher.) Hence, as a consequence of the addition of an alloying element which lowers the melting temperature of the crystal, the curves shown in Fig. 3 are condensed to one single point (c^* , T^*) with $T_g = T_0 = T_i^s$. The instability temperature T_i^s decreases with increasing c and ends in the same triple point where melting and the crystal to glass transition becomes continuous in entropy. Below this triple point the crystal can be destabilized by addition of the alloying element and relaxes to a glass at the extended T_0 line. If the volume change at this condition is also continuous, the crystal-to-glass transformation becomes second order. Hence, the (metastable) equilibrium phase diagram presents three separate regions indicating the stability range of the liquid phase, the glass phase and the supersaturated crystal.

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Pressure-induced amorphization of crystalline silica

R. J. Hemley*, A. P. Jephcoat*, H. K. Mao*,
L. C. Ming† & M. H. Manghnani†

*Geophysical Laboratory, Carnegie Institution of Washington,
2801 Upton Street, NW, Washington, DC 20008, USA

†Hawaii Institute of Geophysics, University of Hawaii,
2525 Correa Road, Honolulu, Hawaii 96822, USA

The crystalline-to-amorphous transformation in the solid state is currently the subject of intense study. With the seminal discovery of amorphization of H₂O ice I under pressure¹, interest has focused on the possible occurrence of pressure-induced amorphization in other systems, the thermodynamic and mechanistic basis of the process, and the relationship between amorphization by pressure and by other means²⁻⁵. Here we report *in situ* synchrotron X-ray diffraction measurements of α -quartz and coesite in a diamond-anvil cell that demonstrate that these crystalline materials transform to amorphous solids at 25–35 GPa and 300 K. We show that an internally consistent thermodynamic description of the process is possible, extending into the pressure domain recent thermodynamic calculations of thermally-induced amorphization of silica polymorphs⁴. The measurements provide constraints on the equations of state and melting relations in this system at high pressures, shed light on the mechanism for glass formation in SiO₂ in laboratory shock-wave experiments and meteorite-impact events, and provide insights into the thermoelastic stability of tetrahedral network structures at high compression.

The transformations between crystalline and amorphous forms of SiO₂ have been extensively studied⁶⁻⁹. In the present experiments, natural (Lisbon) quartz and synthetic coesite⁷ were ground to a grain size of <5 μ m, and loaded into a gasketed diamond-anvil cell of the Mao-Bell design¹⁰. Neon gas was then loaded into the cell to serve as a quasi-hydrostatic pressure-transmitting medium^{11,12}. Energy-dispersive diffraction patterns for α -quartz and coesite at high pressure are shown in Fig. 1. The diffraction pattern from the quartz sample at ~20 GPa (Fig. 1a) consisted of sharp lines that could be readily indexed as those of the crystalline materials (α -quartz and gasket) at high compression¹³⁻¹⁵. Above ~25 GPa, however, the diffraction peaks began to broaden and to decrease markedly in intensity, such that by 30 GPa the contribution from crystalline quartz was very weak. The diffraction pattern of coesite (Fig. 1b) shifted uniformly with pressure to ~24 GPa (refs 16, 17). At 28 GPa small changes in the relative intensities in the peaks at 13–16 keV were noted; these are probably associated with the metastable transition to a distorted coesite structure found in high-pressure Raman measurements². Above 30 GPa, the intensity of the coesite diffraction peaks dropped markedly so that by 34 GPa the silica appeared amorphous by X-ray diffraction. In addition, measurements were performed on lowering the pressure from the maximum of 40 GPa attained in this run to ambient, and no recurrence of sharp diffraction peaks indicative of coesite or any other crystalline phase were observed. Both pressure-quenched samples appeared amorphous by Raman scattering, with spectral features identical to those of densified amorphous silica obtained by compression of silica glass (Herasil) to the same pressure range at 300 K (ref. 2).

The high-pressure X-ray diffraction data demonstrate that on compression at 300 K, both SiO₂ polymorphs lose long-range translational order and become amorphous by X-ray diffraction, rather than transform to stishovite, the thermodynamically stable phase in this P - T range⁶. The previous spectroscopic measurements demonstrated that amorphization of these phases under pressure can be enhanced by the presence of large non-hydrostatic (deviatoric) stresses². The effect was minimized here by

the use of the neon pressure-transmitting medium, which remains a weak solid over the pressure range of these experiments. As a result of the smaller relative volume of the neon in the quartz experiment (Fig. 1), the degree of non-hydrostatic stress was larger in this sample relative to coesite. The Raman study indicated, however, that the transformation in quartz occurs over a similar pressure range (25–30 GPa), even when pressure gradients across the sample become immeasurable². We can rule out the possibility that the transformation is driven by non-hydrostatic effects arising from grain-grain contacts in the present polycrystalline samples³, as the previous spectroscopic experiments showed that the transformation occurs when larger, well-separated single crystals (20–50 μ m) are used.

The P - V relations determined from the present X-ray data are compared with previous measurements for silica phases in Fig. 2. The data indicate that the equations of state for quartz and coesite tend to converge at high pressures, with the amorphization transition occurring in both phases at 17–18 cm³ mol⁻¹, which is significantly larger than the molar volume of stishovite in the same pressure range¹⁸. The recent P - V data to 10 GPa for silica glass measured by Meade and Jeanloz¹⁹ in a diamond-anvil cell using optical techniques at 300 K, are also compared. Constraints on the equation of state of the glass at higher pressures can be obtained from dynamic compression (shock-wave) experiments, which probe the material at high pressures and temperatures. The shock-wave results (uncorrected for temperature) of Wackerle²⁰ and the data from the more recent compilation of Marsh²¹ are plotted in the high-pressure region.

Although the observed transitions are metastable, it is possible to understand the driving force for the amorphization process in a thermodynamic sense by consideration of ambient pressure free-energy data^{22,23} for these phases and the P - V relations at high pressure. Bounds on the observed transformation can be calculated from $\Delta G_{a-b} = \Delta G_{a-b}^0 + \int_0^P \Delta V_{a-b} dP$, where ΔG_{a-b} and ΔV_{a-b} are the free energy and volume differences of phases a and b at constant temperature. The glass is not a thermodynamically stable phase, but its free energy is a useful thermodynamic quantity if the fictive temperature T_f and P - T path history are specified²⁴. In view of the spectroscopic evidence for the similarity in structure of the amorphous form produced from the crystals and the compressed silica glass at 300 K, we assume that the high-pressure amorphous phase is characterized by the available thermodynamic data for the glass. Calorimetric data at 300 K indicate that $\Delta G_{qtz-coes}^0 = 3.80$ kJ mol⁻¹ (ref. 22), and $\Delta G_{qtz-glass}^0 = 7.05$ kJ mol⁻¹ (ref. 23), in which a T_f of 1,480 K (0.1 MPa) was assumed for the glass⁴. With this parameter set, the quartz-coesite transition at 300 K is well predicted (2 GPa) (ref. 6). The free energies calculated from the present data for the two polymorphs remain very close at higher pressures (within the uncertainty of the P - V data). For comparison between the crystalline and amorphous phases, we take the bounds on the hypothetical extrapolated equation of state for the glass shown in Fig. 2. Use of the proposed upper bound on the 300 K isotherm gives equal free energies for the crystalline (both quartz and coesite) and amorphous phases at ~45 GPa. The free energies are equal at ~30 GPa, if the lower bound is used. The latter is close to the pressure at which the observed 'quartz-glass' transformation is complete and the 'coesite-glass' transformation has its onset.

Considering the uncertainties in the P - V data for the glass, the agreement between the observed transformation pressures and the predicted thermodynamic boundaries is close, particularly for the 'quartz-glass' transition. The agreement implies rapid kinetics for the observed amorphization transformations. It is interesting to note that should some overdriving of the transformations occur at 300 K due to sluggish kinetics, with the result that the true (albeit metastable) thermodynamic boundaries for the two transformations are below 30 GPa, the amorphous phase may in fact be more compressible than is indicated

Fig. 1 High-pressure energy dispersive X-ray diffraction patterns of *a*, α -quartz and *b*, coesite samples. The X-ray diffraction was obtained by energy dispersive scattering at a fixed 2θ of $19.90 \pm 0.02^\circ$ using polychromatic synchrotron radiation. The diffraction patterns calculated from the equations of state (Fig. 2), assuming the low-pressure crystalline structure of each phase is preserved at the higher pressures, are indicated along the bottom. Additional peaks in the measured spectra can all be identified as diffraction from the Ne pressure-transmitting medium, from Fe in the T-301 stainless steel gasket used to confine the sample, and from the ruby grains ($\text{Al}_2\text{O}_3:\text{Cr}^{3+}$) used for pressure determination¹¹. The intensities of these secondary diffraction lines tend to change in the spectra as a result of the variability in positioning the sample in the X-ray beam with each increase in pressure. The strong Ne(111) peak in the coesite spectra is due to the larger relative volume of condensed pressure-transmitting gas and to a degree of preferred orientation of the neon crystallites. The neon diffraction lines were also used as a secondary pressure gauge calibrated against the ruby fluorescence scale¹². We note that the coesite sample remained under quasihydrostatic conditions to the maximum pressures (40 GPa). The experiments were performed at beamline A-IV at the Stanford Synchrotron Radiation Laboratory (SSRL), Stanford, California.

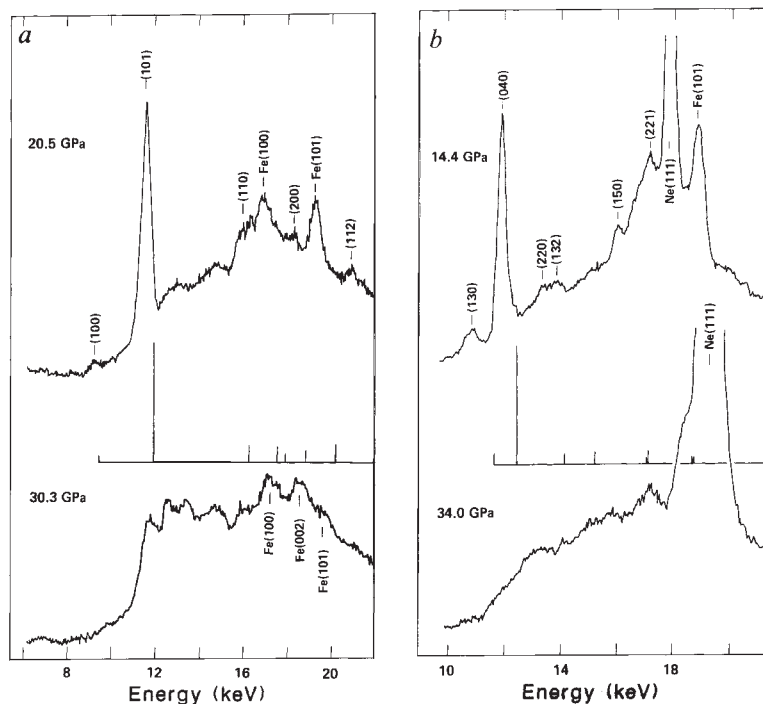
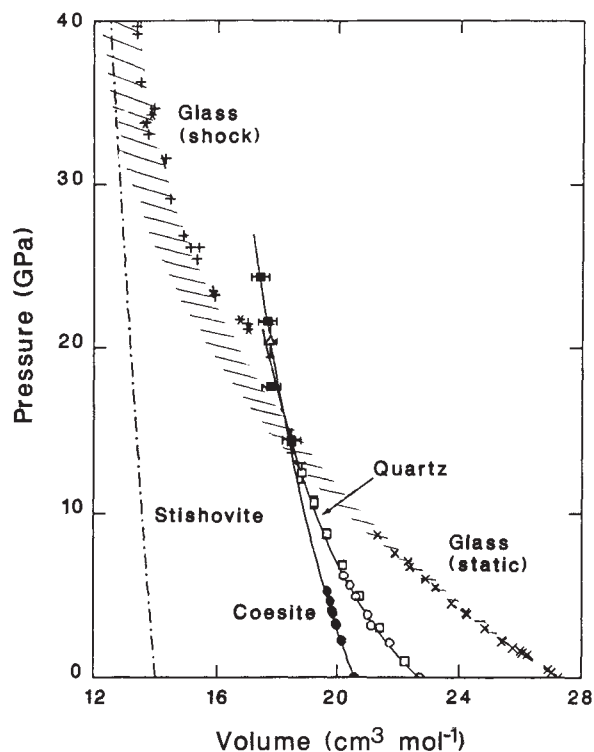


Fig. 2 Pressure-volume equations of state of SiO_2 phases, α -quartz (open symbols): ($\circ$) Levien *et al.*¹⁵; ($\square$) McWhan¹⁴; ($\triangle$) present work. The present data are consistent with the third-order Birch-Murnaghan equation of state determined previously (line) from low pressure data; this equation of state has the parameters: zero-pressure volume $V_0 = 22.71 \text{ cm}^3 \text{ mol}^{-1}$, bulk modulus $K_0 = 37.1 \text{ GPa}$ and bulk modulus pressure derivative $K'_0 = 6.0$ (see ref. 15). Coesite (filled symbols): ($\bullet$) Levien and Prewitt¹⁶; ($\blacksquare$) present data. The coesite equation of state is a third-order Birch-Murnaghan fit to both sets of data, with $V_0 = 20.57 \text{ cm}^3 \text{ mol}^{-1}$ and best-fit parameters $K_0 = 99.8 \pm 2.4 \text{ GPa}$ and $K'_0 = 6.3 \pm 1.2$. The molar volumes were calculated assuming a pseudo-hexagonal structure¹⁶ because of the comparatively low resolution of the energy-dispersive spectra; and the high-pressure phase above 25 GPa was not included. Stishovite: The extrapolated equation of state from Bass *et al.*¹⁸ is shown. Silica glass: ($\times$) Meade and Jeanloz¹⁹ (static, room temperature), where only data from the elastic compression regime and within the hydrostatic limit of experiments ($< 10 \text{ GPa}$) were included. Shock-wave data on silica glass above 20 GPa are also compared: ($*$) Wackerle²⁰; ($+$) Marsh²¹. The shaded region represents proposed bounds on the equation of state of the glass used in the thermodynamic calculations (see text). The equation of state of the glass is difficult to represent with standard formalisms (for example, ref. 19). For the present study it was sufficient to represent the upper and lower bounds by the simple polynomial functions $V = 27.25 + 0.760 P - 0.0131 P^2 + 0.0000690 P^3$, and $V = 27.25 + 0.853 P - 0.0156 P^2 + 0.0000929 P^3$, respectively.



by the lower curve shown in Fig. 2. It is unlikely, however, that the molar volume of the amorphous form is less than that of stishovite, which is the densest known phase of silica and therefore may be taken as an approximate lower bound for the amorphous material (Fig. 2). We also point out that the higher amorphization pressure observed for coesite when compared to the thermodynamic predictions ($\sim 35 \text{ GPa}$ versus 30 GPa) probably results from the fact that coesite undergoes a transformation to a distorted structure before amorphization². The thermodynamic analysis for coesite represents an extension of the recent study of Richet⁴, who considered the thermodynamics of

vitrification of decompressed high-pressure phases. A vitrification temperature of $\sim 875 \text{ K}$ at 0.1 MPa is predicted by Richet for coesite, which, when combined with the present data, provides the first constraints on a metastable 'vitrification boundary' for this SiO_2 phase.

The present results provide new insight into the behaviour of quartz and silica glass under laboratory shock-loading^{9,20} and meteorite impact (see ref. 25 for a recent review). One of the proposed mechanisms by which glass is formed in such processes is quenching from the melt²⁰. The present observations establish that an amorphous solid can also be produced directly by

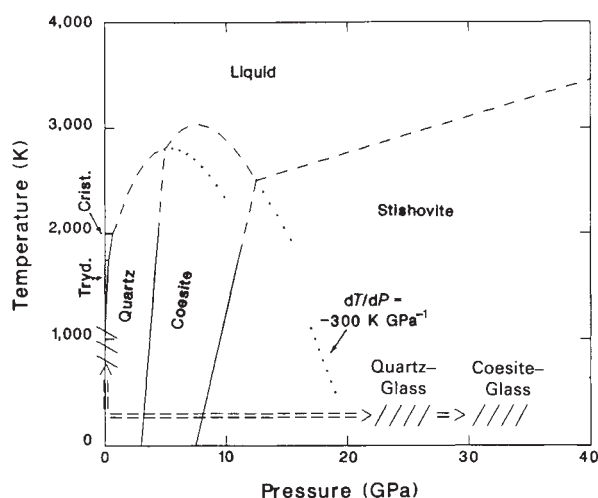


Fig. 3 Phase diagram of SiO₂. The quartz-coesite and coesite-stishovite boundaries are from ref. 24. The quartz and coesite melting curves are those proposed by Jackson²⁷. The stishovite melting curve is constrained by shock-wave data, as discussed by Schmitt²⁵, and the coesite-stishovite-liquid triple point proposed by Davies²⁸. Hypothetical metastable extensions of melting curves of quartz and coesite are indicated by the dotted lines. The horizontal arrow indicates the trajectory of the present pressure-induced amorphization experiment. The vertical arrow shows the thermally-induced amorphization of coesite quenched to 0.1 MPa (in the quartz stability field) predicted recently by Richet⁴. The stability fields of the low-pressure phases cristobalite and trydinite (Sosman⁶) are also shown.

solid-state transformation in the absence of melting (see also ref. 3), in agreement with the conclusions of recent shock-recovery studies²⁶. It has been proposed that at high shock pressures (>20 GPa), quartz and glass enter a mixed phase region in which either crystallization of stishovite or formation of a high-density amorphous phase grows with increasing pressure^{7,20}. Only very small amounts of stishovite, however, have been observed in shock-recovery experiments, a result attributed to the vitrification of the crystalline material during the quench. The thermodynamic analysis of the present data, together with the X-ray diffraction and spectroscopic evidence, suggests that above ~30 GPa the amorphous form can be produced without crystallization at 300 K at densities approaching that of stishovite; it is therefore likely that a high-density amorphous form is produced directly from quartz under shock-loading to these pressures as well.

Pressure-induced amorphization of ice I has been interpreted in terms of the crossing of the metastable extension of its melting curve¹, which has the well known negative Clapeyron slope as a result of the higher density of the liquid relative to ice I. The melting curves of the silica phases at high pressures are poorly constrained owing to the high temperatures involved⁶, but current estimates^{25,27,28} (see Fig. 3) do suggest an isomorphism with H₂O. The sign and magnitude of the ΔV_{tr} for the 'quartz-glass' and 'coesite-glass' transformations are consistent with the negative Clapeyron slopes for the metastable extension of the fusion curves of these crystals, as illustrated by a simple calculation of dT_{tr}/dP for the 'quartz-glass' transition at 18 GPa, where $\Delta V \approx -1.5 \text{ cm}^3 \text{ mol}^{-1}$ (Fig. 2). An accurate calculation of the metastable extension of the melting curves requires information on ΔS_{tr} and the thermal expansion coefficients $\alpha(P)$ at high pressure; assuming a constant α for both phases and a pressure-independent ΔS_{tr} of $5 \text{ J mol}^{-1} \text{ K}^{-1}$ (ref. 24) (in the absence of more data), a high negative value of $dT_{tr}/dP \approx -300 \text{ K GPa}^{-1}$ is calculated (Fig. 3).

For both SiO₂ and H₂O, the temperatures at which pressure-induced amorphization can be made to proceed are low relative to the activation energy barrier for the transformation to the thermodynamically stable phase (that is, 300 K for the silica polymorphs, and 77 K for ice I). The transition from the tetrahedrally coordinated lower pressure SiO₂ phases to stishovite, which has the octahedral coordination of silicon, is extremely sluggish at 300 K (ref. 6). High-pressure vibrational spectra indicate that instead, the SiO₄ tetrahedra distort and gradually breakdown at high pressures (>20 GPa at 300 K) to accommodate more efficient ion packing^{2,29,30}. In view of the evidence for a small thermal activation for the amorphization transition discussed above, it is possible that the process is driven principally by an elastic instability on the crystal potential surface. The negative pressure derivative of the shear modulus C_{66} determined for quartz¹³ supports such a mechanism², giving an extrapolated shear instability in this structure at ~15 GPa. The possibility that elastic instabilities are important in driving the crystal-to-amorphous transition in general is supported by recent *ab initio* studies that find elastic instabilities to be a general feature of classes of ionic crystal structures at high compression^{31,32}, and by recent experimental³³ and theoretical³⁴ studies of neutron-irradiated crystals, which show elastic mode softening before amorphization. Temperature-dependent spectroscopic and diffraction experiments are required to constrain possible mechanisms further. Finally, the similar amorphization behaviour observed in SiO₂ and H₂O, although occurring in different P - T domains, serves as yet another example of the remarkable similarity in physical properties of these two tetrahedrally bonded compounds³⁵.

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Response of northern forests to CO₂-induced climate change

John Pastor* & W. M. Post†

* Natural Resources Research Institute, University of Minnesota, Duluth, Minnesota 55811, USA

† Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831, USA

Climate changes resulting from increases in atmospheric CO₂ are expected to alter forest productivity and species distributions. But forest response to climate change depends in part on changes in soil water and nitrogen availability which limit tree growth. Here we report an investigation into the possible responses of north-eastern North American forests to a warmer and generally drier climate by driving a linked forest productivity/soil process model with climate model predictions corresponding to a doubling of CO₂. The greatest changes occurred at the current boreal/cool temperate forest border. Simulated productivity and biomass increased on soils that retained adequate water for tree growth and decreased on soils with inadequate water. Simulated changes in vegetation composition altered soil nitrogen availability, which in turn amplified the vegetation changes. The simulated responses of the forests were results of a positive feedback between carbon and nitrogen cycles, bounded by negative constraints of soil moisture availability and temperature.

General circulation models suggest a 2–4 °C mean rise in global temperature with CO₂ doubling, with greater warming in higher latitudes than near the equator^{1–5}. This doubling is expected to occur over the next one hundred years, although precise estimates of the doubling time vary according to model assumptions⁶. Several geographically explicit vegetation models suggest profound changes in the distribution of major biomes, particularly in north temperate and boreal regions, as temperature adjusts to this doubling of atmospheric CO₂^{7–10}.

As large fractions of terrestrial biomass are in forests¹¹, it is important to consider the potential responses of these carbon pools to enhance atmospheric CO₂ levels. The differing abilities of soils to hold water have been implicated as an important but poorly understood factor that will control the response of terrestrial ecosystems to CO₂-induced climate change¹². Different tolerances of tree species to drought result in their segregation along moisture gradients in the landscape; this in turn causes different rates of nitrogen and carbon cycling because species differ in growth rates, nitrogen uptake, return in litter, and release of nitrogen and carbon from decomposing litter^{13–16}. The carbon and nitrogen cycles are strongly and reciprocally linked: soil nitrogen availability strongly limits net primary production^{14,17–20} and in turn it is partially controlled by the carbon chemistry, particularly the lignin content, of decomposing litter^{21–23}. Insofar as nitrogen and water are two of the chief limiting factors to forest growth in eastern North America, the responses of trees to drought and the resulting feedbacks between vegetation and soil may control the carbon balance of a large part of the North American continent.

We examined the sensitivity of potential forest responses not only to climate changes which may occur under enhanced CO₂, but also to soil water-holding capacity and the subsequent positive feedbacks that are initiated as a result of changes in the nitrogen cycle. Previous estimates of the transient response of eastern North American forests to CO₂-induced climate changes^{9,10,24,25} have not considered concomitant changes in soil water and nitrogen availability and their interaction with corresponding shifts in vegetation zones.

Rapid changes in photosynthesis and water-use efficiency owing to changes in stomatal control under enhanced CO₂ have been observed in laboratory experiments^{26–31}. But, these physiological processes take place in a context of long-term and large-

scale ecosystem dynamics which pose formidable problems in deriving realistic estimates of forest growth from the few short-term studies now available²⁴. Instead of developing a model to extrapolate from rapid, small-scale physiological processes to ecosystem and global scales, a more feasible approach is to use a quantitative model of forest dynamics appropriate to the scale of a dominant canopy tree (~200 yr and 0.1 ha). At this scale, the effect of fast, single-leaf physiological dynamics is minimized, and competition, climate fluctuations, and plant-soil interactions dominate integration of these rapid single-leaf dynamics into growth of annual rings. This is the scale at which the forest environment, species composition, and associated soil properties change.

Our model simulates the annual establishment, growth, and death of individual trees and decay of cohorts of litter from these trees in a forest plot^{15,16}. Temperature and soil water availability are constraints to population dynamics and their feedback with soil nitrogen availability and light extinction through the canopy. Monthly temperatures and precipitation, mortality, recruitment, and initial tree size vary stochastically about mean values. The model has been extensively validated against independent data of biomass, productivity, species composition, nitrogen cycling, and soil organic matter dynamics in old growth and successional conifer and deciduous forests in eastern North America^{16,32,33}.

Simulations were run for eleven sites using realistic climate projections, on each of two contrasting soil types, to explore the range of potential forest response. We examine in detail the forest response at four sites with initially different climates and different transient responses: (1) Shefferville, northern Quebec (55°N, 66°W; northern interior boreal forest), (2) Kapuskasing, south-central Ontario (49°N, 83°W; southern interior boreal forest), (3) northeastern Minnesota (47°N, 92°W; sub-boreal/prairie border) and (4) northern Maine (47°N, 67°W; maritime subboreal/northern hardwoods).

Current mean monthly temperatures, precipitations, and their standard deviations are from compiled statistics^{34,35}. Changes in monthly temperature with a doubling of CO₂ were calculated by fitting a sine wave through projections for January and July temperatures⁴. Changes in monthly precipitation were calculated by proportioning projected winter and summer changes in precipitation⁴ across the year. Two contrasting soils, sand and silty clay loam, were selected to give estimates of the potential range of soil water availability at each site. Available soil water is assumed to be 18.3 cm m⁻¹ soil depth for the silty clay loam (field moisture capacity at -0.033 MPa is 38.3 cm, wilting point at -1.5 MPa is 20.0 cm) and 10 cm m⁻¹ soil depth for the sand (field moisture capacity, 15 cm and wilting point, 5.0 cm). Each model run began with no vegetation or forest floor present. Initial soil carbon and nitrogen contents were set at 90% of those reported for boreal wet forest and cool temperate moist forest³⁶ to account for the initial lack of a forest floor. Because of the stochastic nature of processes described above, the annual results of 20 model runs were averaged for each soil on each site. This averaged output can be thought of as corresponding to a random sample of 20 stands in a region of homogeneous climate and soils. The model was run for 200 years at each site under current climate, which was then altered linearly over the next hundred years to reach simulated 2 × CO₂ climate for each site⁴, then run for 200 more years with the new climate.

Responses to climatic change at each site are strongly dependent on latitude and changes in the water balance (Table 1, Fig. 1). For far northern Quebec (Fig. 1a–d), the increase in growing degree days was not sufficient to cause simulated boreal forests to be replaced by northern hardwoods. As temperature warmed to optimal levels for spruce, productivity and biomass increased. With climatic warming, there was no decrease in soil water availability on both soils, and therefore only small differences between them in simulated responses of forests.

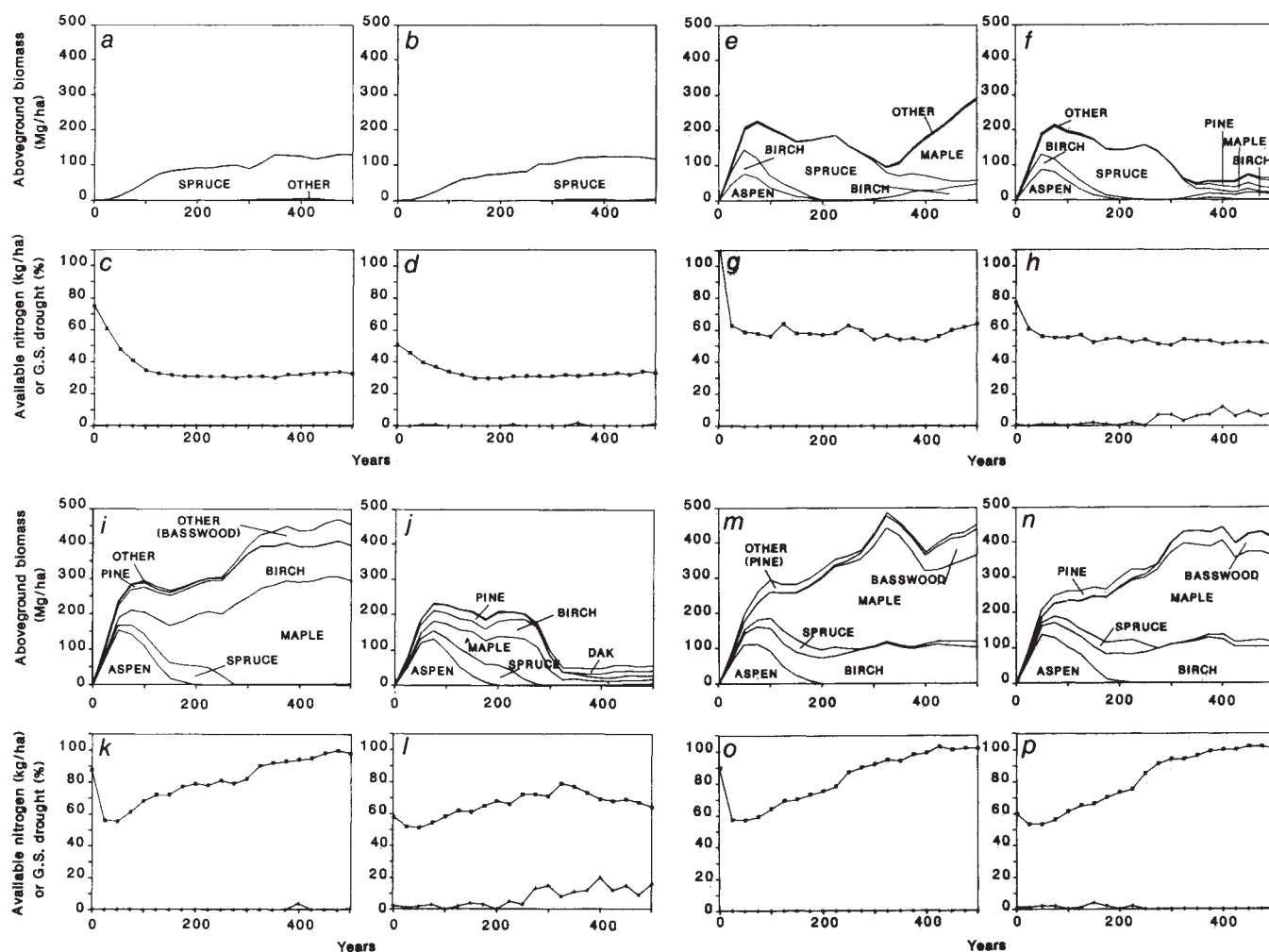


Fig. 1 Simulated changes in biomass, species composition, soil nitrogen availability (square symbols) and the proportion of the growing season (g.s.) that soil water is below wilting point (crossed symbols) for four northern forests, given a doubling of CO_2 . *a-d*, Shefferville, Quebec; *e-h*, Kapuskasing, Ontario; *i-l*, northeastern Minnesota; *m-p*, Maine. *a, c, e, g, i, k, m* and *o*, Silty clay loam soil type; *b, d, f, h, j, l, n* and *p* sand. Horizontal axes represent model runs for 200 years at each site under current climate, which is then altered linearly over the next hundred years to reach simulated $2 \times \text{CO}_2$ climate, then run for 200 more years with the new climate.

The situation is drastically different for those sites in southern boreal and northern hardwood forests, which are located in a band from the Western Great Lakes area eastwards to the Atlantic Ocean (Fig. 1*e-p*). In this area, major shifts in biomes were simulated with warming towards the climatic optima of less cold-hardy species. In most cases, carbon in aboveground biomass changed more than carbon stored in soil (Table 1).

At the current boreal/northern hardwood border, changes in the water balance had a major effect on simulated species composition and carbon balance. In general, on soils where there was no decrease in soil water availability with a doubling of CO_2 , the current mixed spruce-fir-northern hardwood forest was replaced by a more productive northern hardwood forest. This forest was more productive for two reasons. First, in the model, northern hardwoods have a faster intrinsic growth rate and can attain a greater biomass than either spruce or fir. Second, the warmer climate, as well as the higher nitrogen and lower lignin contents of northern hardwood litter, enhance soil nitrogen availability and this enhancement amplifies the effect of warming on productivity. This enhancement of productivity and/or nitrogen availability is particularly noticeable in the simulations for silty clay loams in Ontario (Fig. 1*e* and *g*) and northeastern Minnesota (Fig. 1*i* and *k*) and both soils in Maine (Fig. 1*m-p*).

Where there were increases in the proportion of the growing

season with soil water below wilting point, the simulated mixed spruce-fir/northern hardwood forests were replaced by a stunted pine-oak forest of much lower carbon storage (Fig. 1*f, h, j* and *l*). The most extreme examples are the simulations on sands, where the pine-oak forest under a doubling of CO_2 contains the aboveground biomass characteristic of savannas. But this pattern also occurred to a lesser degree on silty clay loams in northern Wisconsin, southern Michigan, and northern New York (Table 1). Although the warmer temperatures would increase decomposition rate and nitrogen mineralization, the higher lignin and lower nitrogen contents of oak and particularly pine litter compensated for this and reduced decomposition. Generally, as a result, no change or local decreases in soil nitrogen availability occurred.

These simulations assume no direct effect of increased CO_2 concentrations on growth or water use efficiency through changes in stomatal control, which may partially compensate for increased drought²⁶⁻³¹. Although some species more than double their water use efficiency with a doubling of CO_2 (ref. 30), most have a smaller response and others have no response³¹. Some of our simulations forecast three-fold to fourfold increases in the proportion of the growing season during which soil moisture is below the assumed -1.5 MPa wilting point; the reported changes in water-use efficiency under increased CO_2 may not be sufficient to compensate for such large increases in

Table 1 Net changes in climate, soil, and ecosystem properties 200 years after CO₂ doubling ends (year 500) compared with 200-year-old forests under present climate

Site	Latitude	Longitude	Growing season degree days		Growing season drought (%)		Above ground biomass (Mg ha ⁻¹)		Soil organic matter (Mg ha ⁻¹)		Soil nitrogen availability (kg ha ⁻¹)	
			200	500	200	500	200	500	200	500	200	500
North Manitoba	59°N	94°W										
sicl			443	711	0	0	4	89	91	164	23	26
sand			443	711	0	5	2	83	135	195	23	25
North Quebec	55°N	66°W										
sicl			496	727	0	0	93	132	164	207	31	33
sand			496	727	0	1	75	118	190	234	30	33
North West Ontario	54°N	90°W										
sicl			875	1,290	0	0	162	152	280	205	51	40
sand			875	1,290	0	3	128	111	286	236	47	40
Western Ontario	50°N	89°W										
sicl			995	1,515	0	0	164	145	274	156	53	43
sand			995	1,515	0	1	152	131	293	210	53	42
South central Ontario	49°N	83°W										
sicl			1,153	1,716	0	0	179	293	254	306	57	64
sand			1,153	1,716	0	8	142	61	273	268	55	50
North East Minnesota	47°N	92°W										
sicl			1,561	2,095	0	1	292	455	322	419	79	98
sand			1,561	2,095	0	16	211	57	322	309	69	64
North Michigan	47°N	88°W										
sicl			1,611	2,142	0	0	346	357	334	388	80	99
sand			1,611	2,142	0	4	255	206	334	361	72	86
North Wisconsin	46°N	90°W										
sicl			1,780	2,318	0	4	440	248	378	406	89	98
sand			1,789	2,318	0	31	384	11	373	250	87	54
South Michigan	44°N	85°W										
sicl			2,183	2,780	0	7	345	239	328	374	85	92
sand			2,183	2,780	12	36	27	3	210	129	50	32
North New York	45°N	75°W										
sicl			1,991	2,643	0	3	407	277	375	390	93	98
sand			1,991	2,643	8	32	102	23	269	225	63	53
North Maine	47°N	67°W										
sicl			1,606	2,102	0	0	324	456	320	385	75	102
sand			1,606	2,102	0	0	295	417	341	380	73	100

Results are means of 20 model runs for each site in which monthly temperatures and precipitation, tree mortality, and the birth of new trees vary stochastically. Sicl: silty clay loam.

water stress¹⁰. In any case, the current modelling effort provides an ecosystem context to evaluate the direct effects of enhanced CO₂ on tree physiology.

Our results are consistent with current systems theory³⁷. According to this theory, positive feedbacks amplify system responses to changes in negative feedbacks or constraints. Thus, responses of boreal and north temperate forests to CO₂-induced climate change may depend on the balance between changes in the hydrological cycle that constrain the forest response, and the positive feedbacks between the carbon and nitrogen cycles that amplify this response. If the vegetation response to drought decreases nitrogen availability, then the positive feedback between the carbon and nitrogen cycles weakens, resulting in a decline in productivity. Conversely, if climate change alters forest composition, thus enhancing growth of species which can further enhance soil nitrogen availability through the chemistry of their litter, then the same positive feedback results in an increased productivity.

Therefore, interactions between vegetation and water and nitrogen availabilities may produce a bifurcation in the forest ecosystem response, that is, increased productivity where soil water is not limiting and nitrogen availability is enhanced, and decreased productivity where water and nitrogen become more limiting. Forest responses to climate change are as sensitive to the indirect effects of climate and vegetation on soil properties as they are to the direct effects of temperature on tree growth. The heterogeneity of the landscape, particularly the distribution

of various soils, becomes an important factor determining forest responses to climate change, because these bifurcations can occur within, as well as between, biomes.

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The seismic moment budget of slowly spreading ridges

Sean C. Solomon, Paul Y. Huang* & Linda Meinke

Department of Earth, Atmospheric, and Planetary Sciences,
Massachusetts Institute of Technology, Cambridge,
Massachusetts 02139, USA

*Lamont-Doherty Geological Observatory of Columbia University,
Palisades, New York 10964, USA

Mid-ocean ridges with well developed median valleys are commonly the sites of large normal-faulting earthquakes^{1,2}. A recent global study³⁻⁶ of the centroid depths and source mechanisms of such earthquakes has yielded two important conclusions: first, that all the largest mid-ocean ridge earthquakes occur beneath the inner floor of the median valley, and second, that the maximum thickness of the seismogenic layer increases with decreasing spreading rate. These results lend support to the hypothesis that the median valley arises from the necking of a strong, brittle lithosphere under horizontal extension⁷⁻⁹. Here we compare the global rate of seismic moment release along slowly spreading mid-ocean ridges with the rate predicted if widening of the median valley occurs entirely by seismogenic extension of the brittle lithosphere beneath the inner floor. We find that earthquakes account for no more than about 10-20% of the rate of plate separation across the central median valley.

From a formal inversion of long-period teleseismic P and SH waveforms¹⁰, we have determined³⁻⁶ the source parameters of 50 earthquakes during the period 1962-83 along ridges spreading at half rates of 1 to 22 mm per yr¹¹. In addition to centroid depth and source mechanism (double couple orientation, scalar seismic moment, source time function), we have obtained estimates of the water depth above each earthquake epicentre from the predominant period of the water column reverberations in the later portions of the P wavetrains. These estimates are compared in Fig. 1 with the maximum water depth of the median valley inner floor indicated for each epicentral region by maps of the GEMCO¹² series. The agreement between the water depth determined from waveform modelling and that given by the bathymetry, particularly in well surveyed areas, is remarkably good. This figure constitutes the basis for our conclusion that all, or nearly all, of these normal-faulting earthquakes occurred beneath the relatively deep median valley inner floor. We have found no example of a large earthquake beneath the shallower portions of the median valley in the flanking rift mountains.

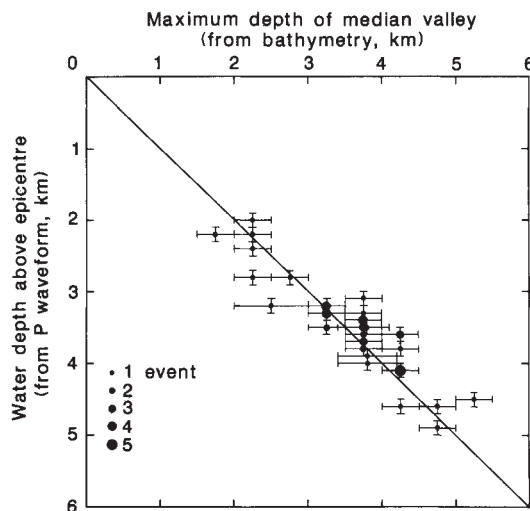


Fig. 1 Water depth above the epicentre of mid-ocean ridge earthquakes estimated from the predominant period of water-column reverberations in long-period P waveforms³⁻⁶ versus the maximum local water depth in the median valley, as indicated on bathymetric maps¹². The uncertainty in the water depth inferred from P waveforms is ± 0.1 km. The range in uncertainty in maximum median valley depth is generally taken to be equal to the bathymetric contour interval. Larger symbols denote more than one event. The water depth inferred from P waveforms falls within ± 0.2 km of the depth interval of the median valley inner floor indicated on bathymetric maps for 90% of the earthquakes.

Geological studies of the inward-facing normal faults that contribute most of the topographic relief of the median valley^{13,14} indicate that the most recently active faults are those making up the inner walls^{15,16}. We suggest that the large earthquakes represented in Fig. 1 occurred by slip on inner wall normal faults and their counterparts at depth beneath the inner floor.

The centroid depths of ridge-axis earthquakes with well constrained source mechanisms are displayed versus local half spreading rate¹¹ in Fig. 2. Centroid depths range from 1 to 6 km, and there is a clear tendency for the maximum centroid depth to shoal with increasing spreading rate. If slip on the fault surface during an earthquake was roughly uniform, then the centroid depth marks the mean depth of seismic faulting. The efficient excitation of water column reverberations by ridge earthquakes³⁻⁶ suggests that fault slip during these events generally extended to the seafloor. Under the assumption that the earthquake fault area was roughly rectangular, then seismic faulting extended to twice the centroid depth for these earthquakes. A brittle layer up to 8-10 km in thickness at half spreading rates of 10-15 mm per yr is indicated for the central median valley (Fig. 2) from this line of reasoning. These thicknesses are in accord with independent estimates of the depth of faulting from median valley microearthquake experiments¹⁷⁻¹⁹ and with estimates of the thickness of the elastic lithosphere at the ridge axis from the spectral relationship between gravity and seafloor topography²⁰⁻²². The maximum thickness of the brittle layer depicted in Fig. 2 is not expected to be applicable to all ridge segments spreading at the indicated rate, but only to those portions which have cooled to the maximum extent before a new major episode of crustal magma injection and volcanism²³.

Several hypotheses have been proposed to explain the existence of a median valley along slowly spreading ridges. In one class of models^{24,25}, median valley topography is a consequence of the loss of hydrodynamic head during upwelling of viscous asthenosphere through a narrow vertical or wedge-shaped conduit. In a second class of models⁷⁻⁹, stretching or necking of the brittle lithosphere rather than viscous forces in the asthenosphere produce the axial valley. Necking is viewed

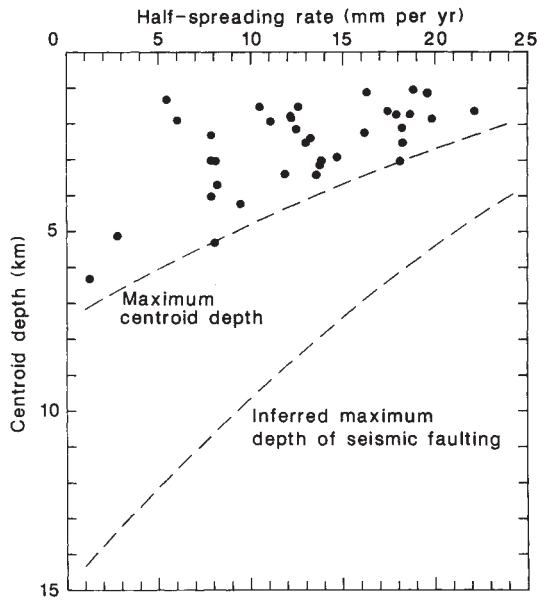


Fig. 2 Centroid depth of mid-ocean ridge earthquakes obtained from body waveform inversion versus local half spreading rate¹¹. Only events with good sampling of the focal sphere for both P and SH waves are included. Formal errors in centroid depths are typically ± 0.2 – 0.4 km, but the true uncertainties range from about ± 0.5 to ± 3 km at 90% confidence⁶. The inferred maximum depth of seismic faulting is equal to twice the maximum centroid depth at each half spreading rate (see text).

as a response to horizontal extension across the plate boundary and is concentrated at the ridge axis because the thickness of the brittle lithosphere is there at its minimum value. The flanking rift mountains, according to these models, are a result of regional isostatic compensation of the median valley⁷ or bending moments produced in an extending strong layer of variable thickness⁹.

The behaviour of ridge-axis earthquakes displayed in Figs 1 and 2 is that predicted by the hypothesis that the median valley along slowly spreading ridges forms by the necking of a strong, brittle lithospheric layer^{7–9}. A simple model of lithospheric extension of the median valley inner floor permits us to compare the predicted rate of release of seismic moment along slowly spreading ridges with the rate observed. Consider a segment of median valley inner floor such as that illustrated in Fig. 3, with a half-width w , a length L along axis, a half spreading rate v , and a thickness h of the brittle layer. Assume that all extension of this layer occurs by seismogenic normal faulting on planar faults extending to depth h and dipping inward at an angle θ . During a time interval t , we suppose that several large ridge-axis earthquakes have occurred and the inner floor has widened to a half-width $w + \Delta w$. Let u_i be the average fault slip during the i th earthquake. Then the accumulated horizontal strain during the interval t is given by $\epsilon_{xx} = \Delta w/w = \cos \theta / w \sum u_i$, and the accumulated seismic moment, including both sides of the median valley, is²⁴ $\sum M_0 = 2\mu Lh / \sin \theta \sum u_i = 2\mu Lwh \epsilon_{xx} / (\sin \theta \cos \theta)$ where μ is the shear modulus. As the rate of horizontal strain ϵ_{xx} is simply v/w , the rate of seismic moment release is

$$\sum M_0 / t = 2\mu Lvh / (\sin \theta \cos \theta) \quad (1)$$

For $\theta \approx 45^\circ$, the fault dip angle indicated by the mechanisms of large earthquakes^{3–6},

$$\sum M_0 / t \approx 4\mu Lvh \quad (2)$$

Note that equations (1) and (2) are independent of the width of the median valley inner floor.

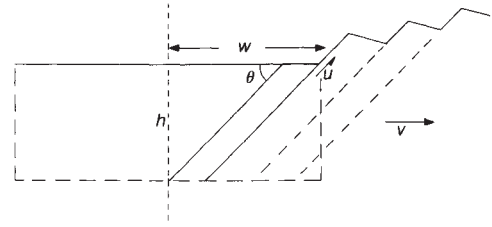


Fig. 3 Simple model for extension of the median valley inner floor by slip on inward-facing normal faults.

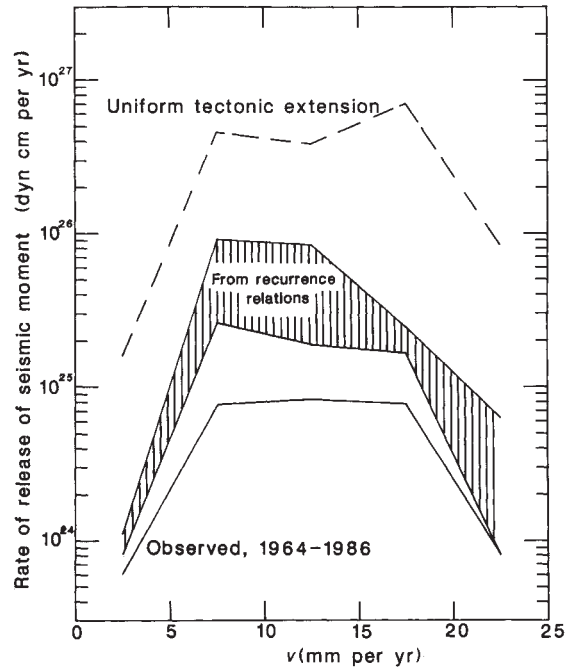


Fig. 4 Rate of release of seismic moment along slowly spreading mid-ocean ridges, grouped by half spreading rate¹¹, v , in intervals of 5 mm per yr. The observed moment release rate, obtained from all normal-faulting ridge-crest earthquakes with known moment, and the rate obtained from empirical recurrence relations are compared with the rate predicted by the simple model of inner floor extension depicted in Fig. 3. The range in moment release rate obtained from earthquake recurrence relations (shaded area) arises from the uncertainty in the critical moment M_c marking a change in the slope of the recurrence rule (see text).

We may use equation (2) to estimate the global rate of seismic moment release for slowly spreading ridges from the total length of all ridge segments and from the average thickness of the brittle layer at each spreading rate v . We have determined $L(v)$ from the global distribution of mid-ocean ridge segments²⁵ and the predicted spreading rate¹¹ along each segment. We assume that the thickness $h(v)$ of the brittle layer is, on average, half the maximum thickness indicated in Fig. 2. With this assumption and with $\mu = 3 \times 10^{11}$ dyn cm⁻², the predicted annual rate of seismic moment release is as shown in Fig. 4 for five equal intervals of half spreading rate between 0 and 25 mm per yr.

The predicted rate may be compared with the observed rate, obtained from a simple sum of the moments of earthquakes with mechanism and moment determined from body waveform inversion^{3–6,26} or from the Harvard centroid-moment tensor inversion catalogues²⁷ along ridge segments in each given interval of spreading rate during the period 1964–1986. As indicated in Fig. 4, the observed rate of moment release is about two orders of magnitude smaller than that predicted from the simple lithospheric extension model of Fig. 3.

A significant component of the seismic moment release, of course, comes from earthquakes too small for detection and

analysis at teleseismic distances. It is common to correct for this contribution under the assumption that the cumulative number N of events per time with moment greater than or equal to M_0 in a region follows a power law of the form

$$\log N(M_0) = A - BM_0 \quad (3)$$

where A and B are constants²⁸. Unfortunately, relation (3) does not appear to hold over the range of observed moments. For teleseismically observed earthquakes, magnitude recurrence relations²⁹ and the magnitude-moment relation³⁰ give a value for B in excess of unity for ridge axis earthquakes, a result that would yield an unbounded contribution to moment release from small earthquakes if it were valid for all moments less than the maximum observed value. In contrast, limited evidence from local experiments along spreading centres suggest that $B \approx 0.5$ is appropriate for microearthquakes^{31,32}. We have calculated the total rate of seismic moment release for each range of spreading rate by fitting equation (3) to available teleseismic data and assuming that a B value of 0.5 holds for events with moments below some critical value M_c . We interpret M_c as marking a transition between larger earthquakes with fault widths constrained by the thickness of the brittle layer and smaller events with source dimensions less than that thickness³³. Comparison of Fig. 2 with estimated fault dimensions for ridge axis earthquakes^{3-6,31,32} suggests that $M_c \approx 10^{23} - 10^{24}$ dyn cm. The observed rate of seismic moment release, so corrected for the contribution from small events, is as much as a factor of 10 greater than the rate of observed moment release without the correction (Fig. 4). Even including this correction, the rate of moment release for this period is at most 3–20% of that predicted by the model of Fig. 3 for ridge systems in each of the five intervals of spreading rate.

We conclude that the rate of seismic moment release by earthquakes along slow spreading mid-ocean ridges is no more than about 10–20% of the rate that should be observed if widening of the median valley occurred entirely by seismogenic extension of the brittle lithosphere beneath the inner floor. This limit is similar to that inferred from the measured throws and dip angles of normal faults in the inner median valley of the Mid-Atlantic Ridge¹³. Most of the rate of plate separation across the mid-ocean ridge inner floor must occur by aseismic processes, including in particular the intrusion of new crust along the central neovolcanic zone. This result provides a constraint on the extent of thinning of the brittle lithosphere permitted by lithospheric necking models⁷⁻⁹ for the formation of the median valley.

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Extra-pair copulation and sperm competition in the zebra finch

T. R. Birkhead, J. Pellatt & F. M. Hunter

Department of Animal Biology, The University, Sheffield S10 2TN, UK

Most birds are monogamous, but recent studies have shown that extra-pair copulations (EPCs) occur frequently^{1,2} despite a range of paternity guards, including mate-guarding and frequent copulation¹. Although EPCs are known to result in extra-pair paternity³⁻⁵, there are no previous quantitative estimates of the success of EPCs in fertilizing eggs. We present here estimates of the likelihood of success of extra-pair copulations in a monogamous passerine, the zebra finch *Poephila guttata*. We show that (1) EPCs occurring under semi-natural conditions in captivity result in extra-pair paternity, (2) sperm from the last male to mate has precedence over previous matings: a single EPC occurring last is disproportionately successful in fertilizing eggs, but EPCs followed by further pair copulations have a low probability of success. These results have important implications for sexual selection theory.

Sperm competition is the competition between spermatozoa of different males to fertilize the eggs of a single female⁶. The potential for sperm competition in birds is high because, unlike most mammals, female birds can store sperm for prolonged periods and ejaculates from different males may occur in the female reproductive tract at the same time^{7,8}. Sperm competition occurs not only among birds with polyandrous mating systems, but also among monogamous species, either when rapid mate replacement occurs⁹, or as a result of EPCs.

The Zebra finch is native to Australia, it breeds colonially and EPCs are known to occur in the wild¹⁰. In captivity, zebra finches copulate about 12 times for each clutch, and females are known to be fertile between day -11 and the day the penultimate egg is laid (where day 0 = the day the first egg is laid) (unpublished data). In the present study the mean clutch was 5.6 eggs $\pm$ 0.86 s.d. ($N = 21$ pairs) and in the wild it is about one egg less than this¹¹.

The present study was conducted with domesticated zebra finches and genetic plumage markers were used to assign paternity; these were grey (G) (wild-type, dominant) and fawn (f) (sex-linked recessive). Only fawn females (fo) were used. These produce all grey offspring when fertilized by homozygous grey (GG) males (Gf sons and Go daughters), and all fawn offspring when fertilized by fawn (ff) males (ff sons and fo daughters). There was no differential fertilizing capacity¹² between homozygous grey and fawn males.

To determine whether EPCs could result in extra-pair paternity we observed seven established pairs (comprising four GG and three ff males paired to fo females) for 72 hours in an aviary (2.4 $\times$ 3.7 $\times$ 2.1 m high) over days -5 to +5 of each pair's reproductive cycle. A total of 23 pair copulations (with cloacal contact) and 31 EPC attempts, seven of which resulted in cloacal

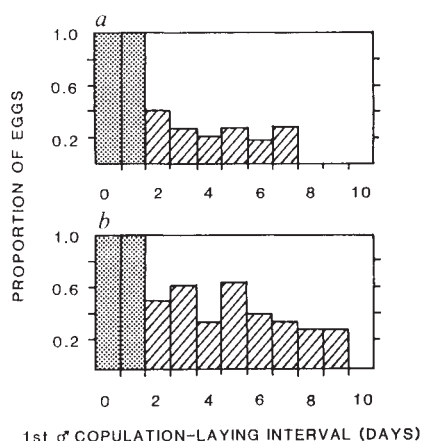


Fig. 1 *a*, Proportion of eggs fertilized by the first (hatched) and second (open) male in experiment 1, in which one male was replaced by another. The two males obtained an estimated 57 and 43% of all copulations, respectively. The x axis shows the number of days between the first male's last copulation and egg-laying. When the interval was 0 or 1 day (stipple), only the first male could fertilize eggs (see ref. 8), so these data are excluded from calculations. The period between days 2 and 10 is when both males could potentially fertilize eggs. Results are from 23 trials with 13 different females; the second male fertilized 74 of 98 (76%) eggs whose paternity we could determine (excluding days 0 and 1). *b*, Proportion of eggs fertilized by the first (hatched) and second (open) males in experiment 2, in which the second male obtained a single EPC on day 0, which was not followed by further pair copulations. Other conventions as in *a*. Data are for 28 trials with 18 different females; the second male fertilized 36 (54%) of 67 eggs whose paternity we could determine (excluding days 0 and 1).

contact, were recorded. Four broods was produced and in one of these extra-pair paternity was detected: a female that was observed to copulate six times with her GG partner and twice with a ff male (on days -2 and -1), produced a brood of two offspring, both of which were fawn. In three further trials, with less detailed observation, extra-pair paternity was detected in one other brood out of 14. Intra-specific brood parasitism was excluded as an alternative explanation: females rarely visited each other's nests during laying, no irregular laying sequences were detected in thrice daily nest inspections, and no obviously different eggs appeared in nests. In total a minimum of 4 (5.6%) out of 71 offspring (2 (11%) out of 18 broods) was produced as a result of EPCs.

These results demonstrate that EPCs can lead to extra-pair paternity. Paired males vigorously attacked males attempting EPC and guarded¹³ their females by close following, and on five occasions forced pair copulations on their females immediately following an EPC; during these copulations the male partner held his female by her head feathers—a behaviour not recorded in any other context.

Two sperm competition experiments were conducted with females in isolated cages; in the first, two males of different plumage types were exchanged about two days (mean -1.8 ± 1.5 s.d., $N = 23$) before the female laid her first egg. Both males were observed to copulate with the female at least once, and the second male always copulated on the day of exchange and at least four hours after the first male. Continuous video recording of 18 trials showed that the first male obtained slightly, but not significantly more copulations (mean $= 5.00 \pm 0.68$ s.e.) than the second male (3.38 ± 0.80 ; paired *t* test, $P > 0.1$). Notwithstanding, the second male fertilized 76% (74 out of 98 eggs) (Fig. 1a), a significantly higher proportion than expected from the ratio (57:43) of copulations (χ^2 , $P < 0.001$).

We conducted the second experiment to determine whether sperm from the last copulation have precedence over sperm

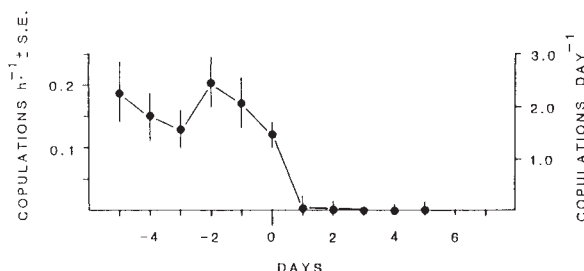


Fig. 2 Temporal pattern of pair copulation in zebra finches maintained as single pairs in cages ($N = 10$ pairs). These data were used to estimate the number of copulations performed by the first male in experiment 2.

from earlier matings. We allowed a female to pair and copulate with a male until she laid her first egg, separated the pair and four hours later allowed the female to make a single behaviourally successful copulation with another male. We then reunited the female with her partner, but separated her from him by a wire screen to prevent further matings. The second male copulated with the female on day 0, and from Fig. 2 we estimated the ratio of copulations by the first and second male to be 9:1. In 28 trials 18 females laid 67 eggs that could have been fertilized by either male: 36 (54%) were fertilized by the extra-pair male (that is, the last copulation) (Fig. 1b). This was a significantly higher proportion than expected from the 9:1 ratio of copulations (χ^2 , $P < 0.001$), but a significantly lower proportion than in the first experiment (χ^2 , $P < 0.01$). To determine whether the lack of complete precedence was due to the failure of some second males to transfer sperm, we paired female zebra finches to vasectomized males and allowed a single copulation with intact males on day -2; 14 (64%) fertile and eight (36%) infertile clutches were produced. If naturally occurring EPCs always result in sperm transfer, we estimate that single EPCs occurring last have a sperm precedence of 84% ($36/67 \times 22/14$).

Previous sperm competition studies with birds¹⁴⁻¹⁶ provided only circumstantial evidence for last male sperm precedence⁸. Moreover, none of these studies provide useful models for considering the probability of a single EPC resulting in offspring. This was because they were either based on an unrealistically small number of (artificial) inseminations, or did not control for the number of copulations performed by each male. In the wild, a paired female is likely to copulate several, or many times with her partner, but only once, or a few times, with an extra-pair male¹.

Our results with zebra finches allow us to estimate the success of single EPCs. An EPC is most likely to be successful if it is the last copulation the female receives; that is, fertilizing between 54 and 84% of eggs. The value of 84% seems more reasonable because it is unlikely that males would seek EPCs if they could not inseminate the female, however, more information on this aspect is required. If the male partner copulates last then the maximum probability of an EPC male fertilizing eggs is between 16 and 46%. Males may therefore compete to be the last to mate with a particular female, and this may explain why birds in which the risks of EPC are high copulate many more times than is necessary to fertilize their eggs¹.

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Guidance of optic nerve fibres by N-cadherin adhesion molecules

Mayumi Matsunaga, Kohei Hatta, Akira Nagafuchi & Masatoshi Takeichi*

Department of Biophysics, Faculty of Science, Kyoto University, Kitashirakawa, Sakyo-ku, Kyoto 606, Japan

The dendritic branches (neurites) of developing neurons migrate along specific pathways to reach their targets. It has been suggested that this migration is guided by factors present on the surface of other neurons or glial cells^{1,2}. The molecular nature of such factors, however, remains to be elucidated. N-cadherin is a cell-surface glycoprotein which belongs to the cadherin family of cell-cell adhesion molecules³. This adhesion molecule is expressed in various neuronal cells as well as in glial cells of the central and peripheral nervous systems in vertebrate embryos^{4–6} and recent immunological studies suggested that N-cadherin may play a role in guiding the migration of neurites on myotubes or astrocytes^{7,8}. To further examine this possibility, we used a molecular-genetic approach; that is, we examined the outgrowth of chicken embryonic optic axons on monolayer cultures of Neuro 2a or L cells transfected with the complementary DNA encoding chicken N-cadherin. The data indicate that N-cadherin is used as a guide molecule for the migration of optic axons on cell surfaces.

The optic nerve fibres of the neural retina of early chicken embryos express N-cadherin^{6,9}. When small fragments of the retina were explanted onto culture dishes coated with collagen gel, they extended neurites onto the substratum. N-cadherin was expressed in these neurites all along their length (Fig. 1d). Antigen binding (Fab) fragments of a rabbit polyclonal antibody against N-cadherin (anti-N-cadherin), which has been shown to block N-cadherin activity⁹, had no effect on the migration or fasciculation of the neurites when added to the cultures, however (data not shown).

We then prepared subconfluent monolayer cultures of Neuro 2a cells, which had little cadherin activity, on cover slips coated neither with collagen nor any other extracellular matrix proteins. When retinal fragments were explanted onto these cultures, they attached to the dishes within a few hours and were surrounded with Neuro 2a cells. In these cultures, the retina extends very few neurites either onto Neuro 2a cells or into cell-free spaces in the dishes (Fig. 2a). We then prepared Neuro 2a cells which express the functional chicken N-cadherin molecules, designated the NN2a-1 line, by transfection with the chicken N-cadherin cDNA joined to a virus promoter (Fig. 1a, c). In dramatic contrast to the previous case, the retina showed a vigorous outgrowth of neurites onto NN2a-1 cell layers (Fig. 2b, d). The tips of these neurites were always in contact with NN2a-1 cells but not with the cell-free substratum (Fig. 2c). The outgrowth of neurites onto NN2a-1 cells was completely inhibited by the Fab of the anti-N-cadherin (Table 1).

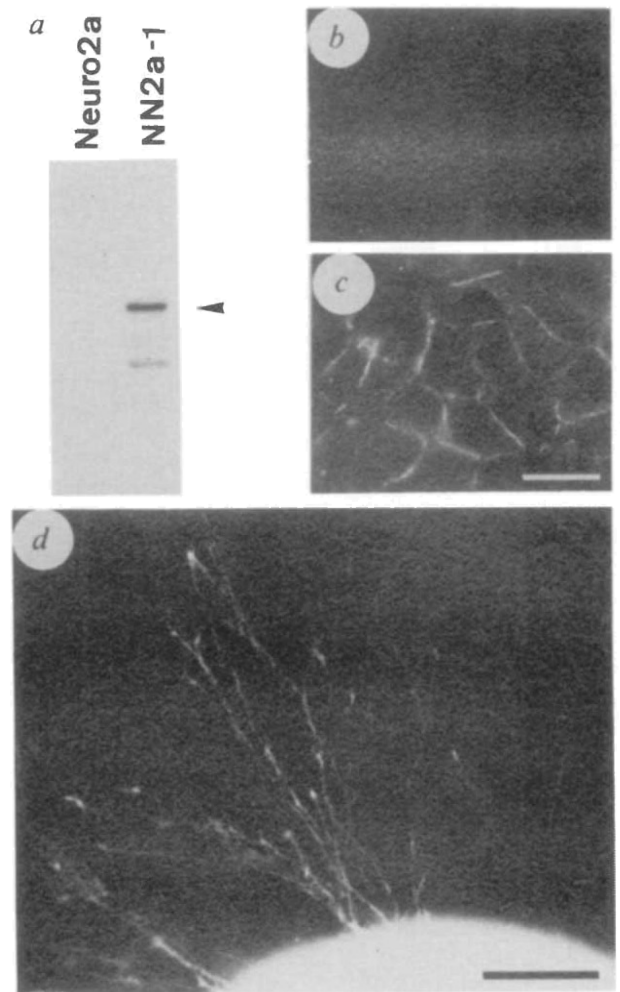


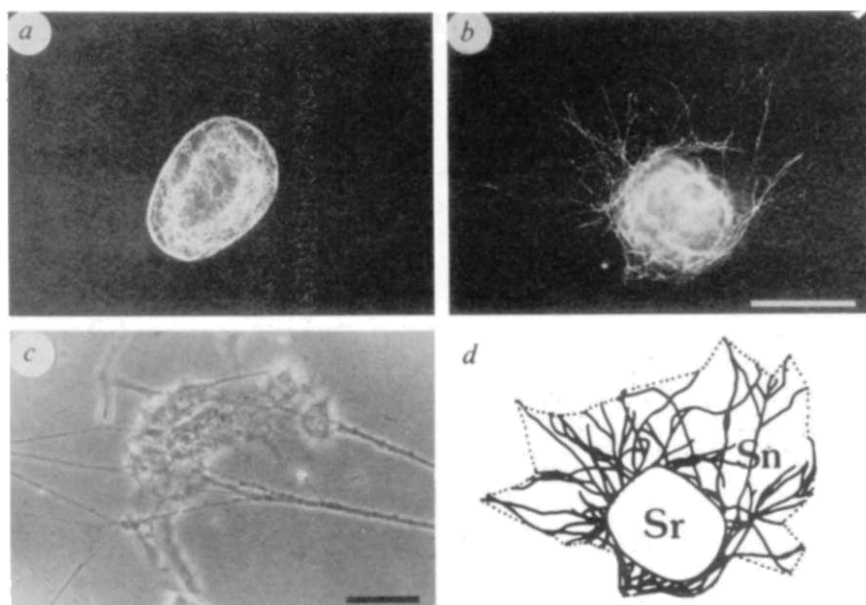
Fig. 1 Expression of N-cadherin in Neuro 2a cells transfected with N-cadherin cDNA and in the optic nerve fibres. *a*, Immunoblot analysis of N-cadherin in the parent Neuro 2a and the transfected line NN2a-1. Arrow head shows the major N-cadherin component of relative molecular mass 132,000. *b* and *c*, Immunofluorescent staining for N-cadherin in Neuro 2a (*b*) and NN2a-1 (*c*) cells. N-cadherin is expressed on the surface of NN2a-1 cells, especially at their boundaries, but not on Neuro 2a cells. Bar, 20 µm. *d*, Immunofluorescent staining for N-cadherin on the optic nerve fibres grown in collagen gel. The retinal body is located at the bottom of the photograph. Bar, 50 µm.

Methods. To introduce the chicken N-cadherin cDNA into Neuro 2a cells²⁸, these cells were co-transfected with pST-Ncad³ and pSTneo²⁹ as described³. Colonies resistant to G418 were isolated and tested for immunoreactivity to the monoclonal antibody NCD-2 to the chicken N-cadherin⁵. One of the positive clones was designated NN2a-1. Cell aggregation experiments showed that Neuro 2a cells had very little endogenous Ca²⁺-dependent aggregating activity, whereas NN2a-1 cells showed Ca²⁺-dependent N-cadherin-mediated aggregating activity. Immunoblot and immunocytochemical analyses were performed using a rabbit anti-N-cadherin serum as described⁹. To allow the outgrowth of optic nerve fibres, small fragments of the neural retina of six-day chick embryos were placed on a Falcon culture dish coated with collagen gel (Cellmatrix Type I-A, Nitta Gelatin) and incubated for three days. Neurites migrating out of the retinal body were fixed and stained immunofluorescently with the rabbit anti-N-cadherin serum as described⁹. The culture medium used throughout the present experiments was a one-to-one mixture of Dulbecco's modified Eagle's medium and Ham's F12 supplemented with 10% fetal calf serum and with 2% heat-inactivated chicken serum.

* To whom correspondence should be addressed.

Fig. 2 Migration of optic nerve fibres on the monolayer cultures of Neuro 2a (*a*) or NN2a-1 cells (*b*). Neurites were visualized by immunofluorescent staining for neurofilaments. Bar, 300 μ m. *c*, Phase-contrast photomicrograph to show neurites attaching onto a colony of NN2a-1 cells. A lower cell density of NN2a-1 cells was purposely used for this particular observation. Under this condition, neurites bridge the gaps between the NN2a-1 colonies. Bar, 50 μ m. *d*, Tracing of neurites and the retinal body on the photograph (*b*), which represents a sample used for the semi-quantitative measurement of the degree of neurite outgrowth described in Table 1. See Table 1 for symbols.

Methods. Cells from these lines were inoculated onto coverslips (3 cm in diameter) placed in 3.5 cm Falcon culture dishes at a density of $5-10 \times 10^5$ cells per dish and incubated for one day. Small fragments of the retina (~ 0.3 mm $\times$ 0.3 mm) obtained from six-day chick embryos were explanted onto these cultures and incubated for another two days. The cultures were then fixed and stained immunofluorescently with the antibody against neurofilaments (Cosmo Bio) by the method described previously⁶.



The same type of experiments were performed using L cells. Retinal fragments extended neurites onto normal L cells to a certain extent. The outgrowth of neurites was significantly promoted, however, when L cells transfected with the chicken N-cadherin cDNA, designated the NL-1 line³, were used as a substratum (Table 1). As a control, we used an L cell line which was transfected with the mouse E-cadherin cDNA, designated EL-8 (ref. 10), as a substratum. The neurites migrated onto the EL-8 cells to the same degree as onto the untransfected L cells (Table 1).

These results demonstrated that the optic axons recognize N-cadherin expressed on the surface of other cells and use it as a guide molecule for their migration. Our previous studies suggested that cadherins interact with other cadherin molecules in a homophilic manner to bind cells^{10,11}. The interaction between N-cadherin molecules present on neurites and those present on cells used as a substratum, therefore, must enhance the adhesion between these heterotypic cells, and consequently supports the migration of neurites. Perhaps, in this adhesion system, the optic axons specifically recognize N-cadherin but

not other subclasses of cadherin, as we recently obtained evidence that cadherins preferentially interact with the same subclass (A. Nose and M.T., in preparation). This idea is also supported by the present finding that the mouse E-cadherin, which has 49% similarity in amino-acid sequence to the chicken N-cadherin³, has no effect on neurite migration. The specificities of cadherin subclasses may thus be important in the path-finding mechanisms of neurite migration. This possibility should be tested by comparing different cadherins derived from the same species of animals in future studies.

The question of whether N-cadherin is used for the neurite-neurite adhesion may be raised. The present observations indicated that the fasciculation of neurites was not obviously affected by the anti-N-cadherin Fab. This suggests that other molecules such as NgCAM¹², L1¹³, G4^{14,15} play a more important role in neurite fasciculation. It is also possible that there is a mechanism which inhibits the interaction between N-cadherin molecules on the neurites.

Although the outgrowth of neurites can be supported by various extracellular matrix substances, such as laminin,

Table 1 Outgrowth of optic nerve fibres on various cell monolayers

Cell lines (cadherin type)	Neurite extension index (S_n/S_r)					Number of explants examined
	0-0.5	0.5-1	1-2	2-4	4-8	
Neuro 2a	20 (100)					20
NN2a-1 (N)			7 (35)	8 (40)	5 (25)	20
NN2a-1 + Fab	9 (100)					9
L	8 (34.8)	2 (8.7)	3 (13.0)	8 (34.8)	2 (8.7)	23
NL-1 (N)					4 (22.0)	18
EL-8 (E)	7 (29.2)	5 (20.8)	6 (25.0)	5 (20.8)	1 (4.2)	24

Degree of neurite outgrowth was represented by the neurite extension index, defined as follows. As shown in Fig. 2*d*, the outlines of the area with neurite outgrowth (S_n) and that containing the retinal body (S_r) were traced on transparent paper. The ratio of these two areas, S_n/S_r , was obtained by weighing the paper corresponding to each area, and this is defined as the neurite extension index. The values indicate the number of explants which are grouped into each category of the index, and the percentage with respect to the total number is shown in parentheses. Monolayers of cell lines were prepared as described in Fig. 2, except that L and its derivatives (NL-1 and EL-8) were inoculated at a density of $1-2 \times 10^5$ per dish. After explanting retinal fragments, they were cultured with or without 1.8 mg ml⁻¹ Fab fragments of the rabbit anti-N-cadherin⁹. After two days, the explants were fixed, stained for neurofilaments and photographed; finally their shape was traced.

fibronectin and collagen¹⁶⁻²⁰, it has been shown that the surfaces of certain cell types, such as astrocytes and myotubes, also provide important factors for axonal guidance²¹⁻²⁴. The present findings strongly suggest that one of these factors is N-cadherin. This view is consistent with the previous observations that some types of glial cells or myotubes express N-cadherin^{6,11} and that the migration of neurites onto these cells can be inhibited by antibodies against N-cadherin^{7,8}. In the optic pathway, the growth cones of migrating retinal ganglion cells are in a position to contact neuroepithelial cells but not extracellular basal lamina²⁵⁻²⁷, suggesting that the surface of neuroepithelial cells is directly involved in guiding the neurites. Because N-cadherin is expressed in neuroepithelia of early embryos⁶, it is highly probable that this molecule actually plays a role in axonal guidance in the central nervous system.

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Highly cooperative feedback control of retinal rod guanylate cyclase by calcium ions

Karl-Wilhelm Koch & Lubert Stryer

Department of Cell Biology, Sherman Fairchild Center, Stanford University School of Medicine, Stanford, California 94305, USA

Visual excitation in retinal rod cells is mediated by a cascade that leads to the amplified hydrolysis of cyclic GMP (cGMP) and the consequent closure of cGMP-activated cation-specific channels in the plasma membrane¹⁻³. Recovery of the dark state requires the resynthesis of cGMP, which is catalysed by guanylate cyclase, an axoneme-associated enzyme⁴⁻⁶. The lowering of the cytosolic calcium concentration (Ca_i) following illumination⁷⁻¹⁰ is thought to be important in stimulating cyclase activity^{11,12}. This hypothesis is supported by the finding that the cGMP content of rod outer segments increases several-fold when Ca_i is lowered to less than 10 nM¹³⁻¹⁶. It is evident that cGMP and Ca_i levels are reciprocally controlled by negative feedback^{1,2}. Guanylate cyclase from toad ROS is strongly stimulated when the calcium level is lowered from 10 μ M to 10 nM, but only if they are excited by light¹⁷. We show here that the guanylate cyclase activity of unilluminated bovine rod outer segments increases markedly (5 to 20-fold) when the calcium level is lowered from 200 nM to 50 nM. This steep dependence of guanylate cyclase activity on the calcium level in the physiological range has a Hill coefficient of 3.9. Stimulation at low calcium levels is mediated by a protein that can be released from the outer segment membranes by washing with a low salt buffer. Calcium sensitivity is partially restored by adding the soluble extract back to the washed membranes. The highly cooperative activation of guanylate cyclase by the light-induced lowering of Ca_i is likely to be a key event in restoring the dark current after excitation.

The guanylate cyclase activity of bovine rod outer segment (ROS) membranes is highly dependent on the concentration of calcium ions in the 20-200 nM range (Fig. 1a). The specific activity decreased from 11 to 2 nmol per min per mg rhodopsin

when the free calcium concentration ($[Ca^{2+}]$) was raised from 20 to 120 nM. The change was even larger in some preparations (such as in Fig. 2). Basal activities as low as 0.6 nmol min⁻¹ mg⁻¹, and stimulated activities as high as 20 nmol min⁻¹ mg⁻¹, were observed. The change in cyclase activity was half-maximal at 90 nM Ca^{2+} . A Hill plot of the calcium dependence of cyclase activity is shown in Fig. 1b. The average slope of Hill plots of five independent sets of data is 3.9 ± 1.2 (s.d.), indicating that regulation by calcium ions is a highly cooperative process. Illumination resulting in the formation of 0.05% photoexcited rhodopsin had no effect on cyclase activity, either at 63 or 186 nM Ca^{2+} .

Calcium sensitivity was lost when ROS membranes were washed with a large volume of a low salt buffer (Fig. 2). The cyclase activity of these washed ROS was low (<1 nmol min⁻¹ mg⁻¹) irrespective of the concentration of calcium ion. This basal cyclase activity is tenaciously bound to the membranes. Addition of a concentrate of the pooled supernatants (produced by centrifugation through a Centricon microconcentrator or by high-speed vacuum evaporation) to washed ROS did not restore their calcium sensitivity at either low or moderate ionic strength. In contrast, calcium sensitivity was partially restored (to 62% of that of native ROS) when the soluble fraction derived from washing ROS with an equal volume of the low salt buffer was added back to the membranes (Fig. 3). This supernatant was devoid of measurable cyclase activity (<1% of that present in the pellet). Digestion of this soluble fraction by protease from *Streptomyces griseus* destroyed its capacity to stimulate cyclase, indicating that the active component is a protein. The addition of brain calmodulin had no effect on the cyclase activity of washed or native ROS at either low or high $[Ca^{2+}]$. Likewise, trifluoperazine and W-7, which inhibit calmodulin-mediated processes, had no effect on catalytic activity. Thus, the calcium-sensitive protein is probably different from calmodulin.

These experiments lead to three conclusions: (1) Retinal rod guanylate cyclase is regulated by a highly cooperative calcium switch that senses changes in $[Ca^{2+}]$ in the submicromolar range. (2) A membrane-bound protein that can be released at low ionic strength is an essential component of this switch. (3) Guanylate cyclase in the absence of the calcium-sensitive regulator has low activity, irrespective of the calcium level. Hence, guanylate

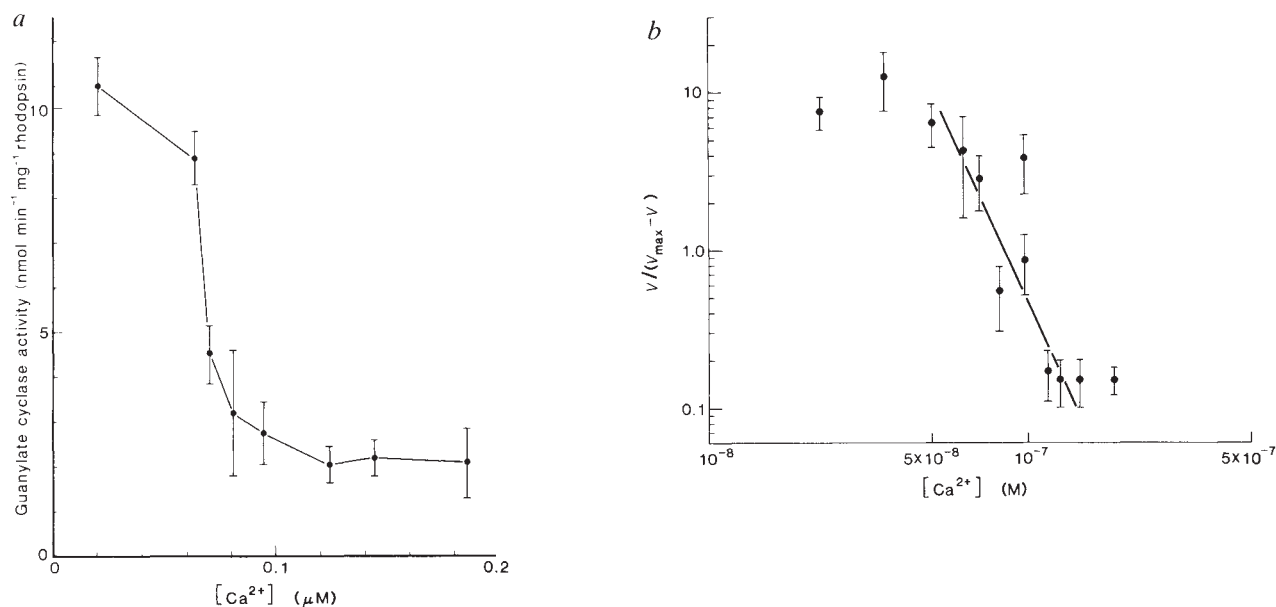


Fig. 1 Regulation of guanylate cyclase activity by calcium ions. *a*, Dependence of enzymatic activity on the concentration of free Ca^{2+} . *b*, Hill plot of the calcium dependence of enzymatic activity. The slope is 4.4. The results of three sets of experiments in *a* and five in *b* are shown. Standard deviations are marked by error bars.

Methods. Retinas were removed from fresh bovine eyes under dim red light and shaken for 1 min in buffer A (115 mM NaCl, 2.5 mM KCl, 1 mM MgCl_2 , 10 mM HEPES pH 7.5) containing 35% (w/w) sucrose. Nearly the same results were obtained with frozen retinas. This suspension was centrifuged for 10 min at 6,000 r.p.m. (4,000g), the pellet was discarded, and the supernatant was diluted 1:2 with buffer A. The ROS suspension was centrifuged again for 10 min at 4,000 r.p.m. (1,900g) and the pellet was resuspended in a 4 mM MOPS pH 7.1, 5 mM dithiothreitol at a final rhodopsin concentration in the range 0.08–0.15 mM. Guanylate cyclase activity was determined by a modification of a previously thin-layer chromatography (TLC) assay^{5,17}. Five μl of ROS suspension (containing 16–30 μg rhodopsin) were mixed with 10 μl of assay buffer and 10 μl of a Ca^{2+} -EGTA solution. The final reaction mixture contained 40 mM MOPS pH 7.1, 56 mM KCl, 8 mM NaCl, 12 mM MgCl_2 , 2 mM EGTA, 2 mM [^{32}P]GTP (specific activity of 20–40 mCi mmol^{-1}), 1.6 mM [^3H]cGMP (specific activity of 0.7 mCi mmol^{-1}), and 2 mM 3-isobutyl-1-methylxanthine (IBMX) (to inhibit phosphodiesterase activity). Different concentrations of free Ca^{2+} in the reaction mixtures were obtained by varying the ratio of added Ca^{2+} to EGTA, which was held constant at 2 mM. The free Ca^{2+} concentration in the assay medium was determined using Fura-2, a fluorescent calcium indicator²⁵, taking K_d to be 224 nM. The following Ca^{2+} :EGTA ratios (estimated free [Ca^{2+}] in parentheses) were used: 0.9 (186 nM), 0.5 (144 nM), 0.4 (130 nM), 0.3 (114 nM), 0.2 (96 nM), 0.15 (81 nM), 0.125 (70 nM), 0.1 (63 nM), 0.07 (52 nM), 0.03 (35 nM). Samples with EGTA but no added calcium ion contained about 20 nM [Ca^{2+}]. The reaction was stopped after 5–10 min by adding 5 μl of 100 mM EDTA and boiling for 2 min. All of these steps were carried out in complete darkness at room temperature. The boiled assay mixtures were centrifuged and nucleotides were analysed either by TLC or high-pressure liquid chromatography (HPLC). Four μl aliquots of the clear supernatants were spotted on a polyethyleneimine cellulose TLC sheet, which was developed first with distilled water and then with 0.2 M LiCl. GTP, GDP, 5' GMP, guanosine and cGMP were added as standards. The cGMP spots detected under UV light were cut out and the nucleotide was eluted into scintillation vials by adding 0.3 ml of 25 mM MgSO_4 . Ten ml of NEN scintillation liquid was added to each vial and the content of ^{32}P and ^3H was measured in a Beckman scintillation counter. Alternatively, nucleotides in the reaction mixture were analysed by reverse phase HPLC²⁶ on a 4.6 $\times$ 10 mm Adsorbosphere 7 μm C_{18} bead (Alltech) column. For HPLC assays, the total reaction volume was scaled up to 60–100 μl and radioactive nucleotides were not used. Fifty μl of the clear supernatant was injected onto the HPLC column, which was equilibrated with 100 mM KH_2PO_4 pH 3.0. Nucleotides were eluted in 10 min with a linear gradient beginning with this buffer and ending with 75% methanol containing 25 mM KH_2PO_4 pH 3.0. Guanylate cyclase activities determined by HPLC analysis agreed well with those determined by TLC analysis. Enzymatic activities were corrected for the hydrolysis of cGMP as monitored by the destruction of [^3H]cGMP. About 20% of the cGMP present was hydrolysed during the 5–10 min incubation. The amount of cGMP formed was linear with time over 10 min.

cyclase is stimulated by this regulatory protein at low [Ca^{2+}] rather than being inhibited by it at high [Ca^{2+}].

Our results with bovine ROS differ significantly from those of Pepe *et al.* with toad ROS¹⁷. They found that the guanylate cyclase activity of unilluminated toad ROS was independent of [Ca^{2+}] from 10 nM to 1 μM . In contrast, the cyclase activity of their illuminated ROS was much higher in 10 nM than in 1 μM [Ca^{2+}]. Their observed calcium dependence was much less steep than ours. It will be important to determine the reasons for these differences. The isolation and characterization of the calcium-sensitive protein are additional challenges. The high degree of cooperativity exhibited by this regulatory component makes it especially interesting. This protein is also intriguing because its stimulatory effect is switched off by elevated [Ca^{2+}], the reverse of that found for calmodulin and other previously described calcium-sensitive proteins.

The switching of guanylate cyclase activity by calcium ions observed here is likely to be physiologically significant. First, switching occurs at 90 nM [Ca^{2+}]. Electrophysiological and optical studies of intact amphibian retinal rod cells suggest that Ca_i drops from more than 250 nM to less than 70 nM within a second after a light pulse^{8,9,18,19}. Mammalian rods, like amphibian rods, contain a potent sodium-calcium antiporter²⁰. Hence, it seems likely that the calcium level in mammalian rods also drops markedly following light-triggered closure of the cGMP-gated channel. Second, the cyclase activity of these ROS suspensions is in accord with cGMP turnover rates in intact retinas as measured by ^{18}O incorporation into guanine nucleotides²¹. The ^{18}O studies of intact rabbit retinas showed that the cGMP flux increases from 12 to 67 $\mu\text{M s}^{-1}$ (in the ROS volume) on illumination. Our basal cyclase activity of 1 $\text{nmol min}^{-1} \text{mg}^{-1}$ corresponds to 4 $\mu\text{M s}^{-1}$, and our stimulated activity of 15 nmol

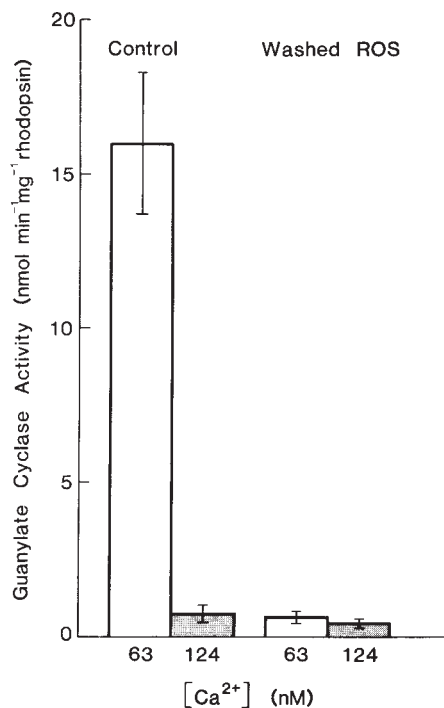


Fig. 2 Loss of calcium sensitivity following the washing of ROS membranes with a large volume of low salt buffer. *a*, Guanylate cyclase activity of the unwashed ROS suspension at 63 and 124 nM [Ca²⁺]. *b*, Two ml of 10 mM MOPS pH 7.1 were added to 50 μ l of ROS membranes containing 100 μ M rhodopsin. The suspension was centrifuged at 18,000 r.p.m. (39,000g) for 30 min. The pellet was resuspended in 50 μ l of 10 mM MOPS pH 7.1 and assayed for guanylate cyclase activity. Assay mixtures as described in Fig. 1.

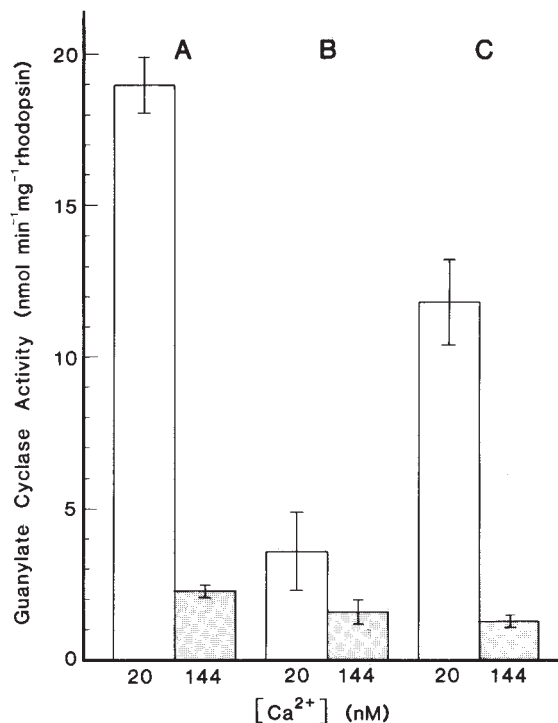


Fig. 3 Partial restoration of calcium sensitivity by addition of a soluble extract to washed ROS membranes. *a*, Guanylate cyclase activity of the unwashed ROS suspension at 20 and 144 nM [Ca²⁺]. *b*, Fifty μ l of 10 mM MOPS pH 7.1 were added to 50 μ l of ROS membranes containing 100 μ M rhodopsin. The mixture was centrifuged in an airfuge (150,000g) for 5 min. The supernatant was devoid of cyclase activity. The pellet contained cyclase activity but exhibited little calcium sensitivity. *c*, The supernatant from (*b*) was added to the pellet. Much of the calcium sensitivity found in (*a*) was restored. The assay mixtures were as described in Fig. 1.

min⁻¹ mg⁻¹ corresponds to 60 μ M s⁻¹ (calculated for a rhodopsin concentration of 6 mM in disks, which occupy half the volume of an outer segment). The stimulated cyclase activity of our ROS suspension seems sufficient to replenish the cGMP store following illumination¹.

A coherent picture of the recovery process is emerging^{1,2,22}. In the dark, the entry of calcium ions through the cGMP-gated cation-specific channels in the plasma membrane is balanced by efflux of calcium through the calcium-sodium antiporter to give a Ca_i of 300 nM⁷⁻¹⁰. Light leads to the hydrolysis of cGMP and the closure of channels. The influx of calcium is blocked, but its efflux through the antiporter continues, which results in a drop of Ca_i to less than 70 nM. This decrease in Ca_i results in the stimulation of guanylate cyclase by a calcium-sensitive protein. The consequent formation of cGMP restores the supply

of this messenger and reopens the channels. The entry of calcium then leads to decreased activity of guanylate cyclase and the return to the dark state. This feedback loop involving calcium ions is likely to be a major contributor to the maintenance of a constant cGMP level in the dark and to its recovery following illumination. It will be interesting to learn whether calcium acts at additional sites, such as the cGMP phosphodiesterase^{23,24}. The experiments reported here showing that guanylate cyclase is exquisitely sensitive to calcium ions in the physiological range strengthen the hypothesis that calcium ions play a key role in the recovery process following visual excitation.

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Photoreceptor light adaptation is mediated by cytoplasmic calcium concentration

H. R. Matthews*, R. L. W. Murphy*, G. L. Fain† & T. D. Lamb*

* Physiological Laboratory, University of Cambridge, Downing Street, Cambridge CB2 3EG, UK

† Department of Ophthalmology, The Jules Stein Eye Institute, UCLA School of Medicine, Los Angeles, California 90024, USA

The vertebrate visual system can operate over a large range of light intensities. This is possible in part because the sensitivity of photoreceptors decreases approximately in inverse proportion to the background light intensity¹⁻³. This process, called photoreceptor light adaptation, is known to be mediated by a diffusible intracellular messenger⁴⁻⁶, but the identity of the messenger is still unclear. There has been considerable speculation that decreased cytoplasmic Ca^{2+} concentration (Ca_i^{2+}) may play a role in light adaptation⁷⁻¹¹, and recent experiments in which Ca^{2+} buffer was incorporated into rod-cells^{10,11} have supported this notion. The extent of the contribution of calcium, however, remains unresolved. We now show that light-dependent changes in sensitivity in amphibian photoreceptors can be abolished by preventing movements of Ca^{2+} across the outer-segment plasma membrane. These experiments demonstrate that light adaptation in photoreceptors is mediated in cones primarily, and in rods perhaps exclusively, by changes in Ca_i^{2+} .

In darkness a steady Ca^{2+} influx into the photoreceptor outer segment through the light-dependent channels¹²⁻¹⁴ is balanced by an equal efflux of Ca^{2+} by Na^+ - Ca^{2+} exchange¹⁵⁻¹⁷. Light closes the channels, and the continued extrusion of Ca^{2+} produces a decrease in Ca_i^{2+} (refs 8, 18-20). In an attempt to prevent light-induced changes in Ca_i^{2+} , we minimized Ca^{2+} influx by reducing the extracellular free- Ca^{2+} concentration to 0.3-3 μM ,

and we minimized Ca^{2+} efflux by replacing Na^+ with another permeant cation which does not support Na^+ - Ca^{2+} exchange (guanidinium²¹ in most experiments). To compare responses in Ringer and in low- Ca^{2+} /zero- Na^+ solution, we rapidly stepped^{14,16,22} the boundary between the two flowing solutions across the outer segment of a salamander rod- or cone-cell.

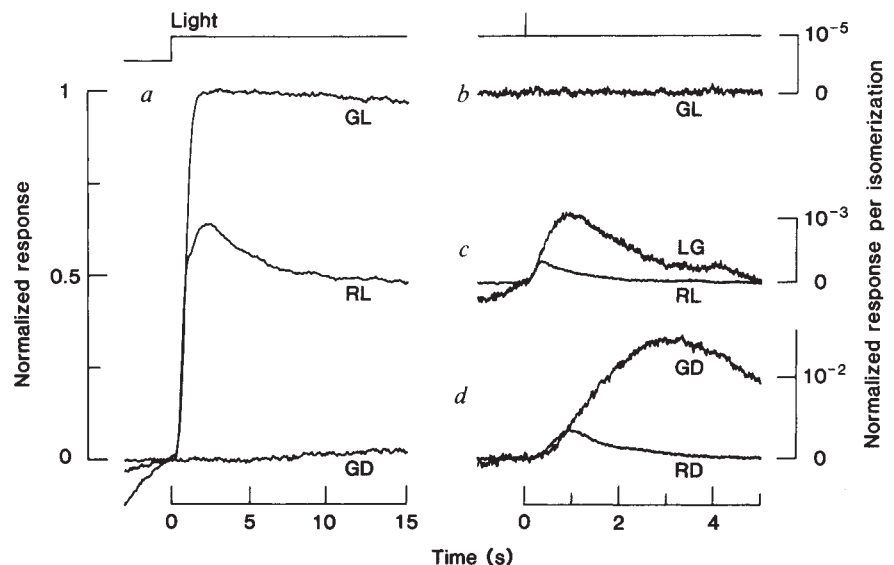
Representative responses from a rod for a single background light intensity are shown in Fig. 1. For a given stimulus (steady illumination *a*, or incremental flashes, *c*, *d*) the early rising phase is similar in Ringer and in low- Ca^{2+} /zero- Na^+ solution. During exposure to low- Ca^{2+} /zero- Na^+ solution, however, the responses continue to rise for a longer time, and are therefore larger and (for subsaturating intensities) reach peak amplitude later. Similar behaviour has previously been reported when calcium buffer was incorporated into the rod cytoplasm¹⁰⁻¹¹, a procedure also expected to slow changes in Ca_i^{2+} . These results indicate that when Ca_i^{2+} is prevented from falling, the normal rapid recovery of the light response is also prevented.

Comparison of Fig. 1*b* and 1*c* shows that the order of presentation of the low- Ca^{2+} /zero- Na^+ solution and the background illumination is important. When the low- Ca^{2+} /zero- Na^+ solution was presented first, the elimination of the circulating current by the relatively dim background (*a*) had the inevitable consequence that even a very bright superimposed flash produced no additional response (*b*). On the other hand, when the low- Ca^{2+} /zero- Na^+ solution was presented after the rod had adapted to steady illumination, a substantial response was obtained with a dim test flash (Fig. 1*c*, trace LG).

Figure 2 summarizes results from experiments like that shown in Fig. 1, for a number of different background intensities. The amplitude of the response to steady illumination (Fig. 2*a*) increases gradually as a function of background intensity in Ringer (●), but increases more steeply and reaches half-saturation at a substantially lower intensity for backgrounds presented during exposure to low- Ca^{2+} /zero- Na^+ solution (○). The curve fitted to the open symbols is the exponential saturation-equation, $1 - \exp(-kI_B)$, used previously to describe the amplitude at fixed early times during the flash response in normal

Fig. 1 Normalized suction-pipette current from a salamander rod in response to a representative intensity of steady illumination (*a*) or superimposed flashes (*b-d*). Traces are identified as: D, in darkness, or L, in steady light (8.1 photons $\mu\text{m}^{-2} \text{s}^{-1}$); and R, in Ringer, or G, during exposure to low- Ca^{2+} /zero- Na^+ solution containing 3 μM free- Ca^{2+} and 110 mM guanidinium. Panel (*b*) shows the failure of an intense flash (410 photons μm^{-2}) to elicit any response when steady illumination was presented during exposure to low- Ca^{2+} /zero- Na^+ solution (trace GL). Panel (*c*) illustrates the response to much dimmer flashes (4.5 photons μm^{-2}) presented on the same background, either in Ringer (trace RL) or when the low- Ca^{2+} /zero- Na^+ solution was presented after equilibration to the background light (trace LG). Panel (*d*) illustrates responses to still dimmer flashes (0.6 photons μm^{-2}) in darkness. All responses have been normalized with respect to dark current (~ 37 pA in Ringer and ~ 33 pA in low- Ca^{2+} /zero- Na^+). Scale bars for (*b-d*) indicate sensitivity in units of normalized response per photoisomerization. Exposures to low- Ca^{2+} /zero- Na^+ began 5 s before time zero in (*a*). An initial sloping baseline as in traces GL (*a*) and LG (*c*) was often seen during the first few exposures to low- Ca^{2+} , zero- Na^+ solution.

Methods. Photoreceptors were isolated from the retina of the larval salamander *Ambystoma tigrinum* and the circulating current was recorded with a suction pipette^{10,22}. Flowing Ringer⁶ or low- Ca^{2+} /zero- Na^+ solution could be rapidly stepped across the outer segment^{14,16,22}. In most experiments the permeant cation replacing external Na^+ was guanidinium²¹, but comparable results were obtained using Li^+ , which has also been shown not to support Na^+ - Ca^{2+} exchange¹⁵⁻¹⁷. For rods we buffered the extracellular calcium concentration (Ca_o^{2+}) to 3 μM with EDTA-Mg, as in most cells the dark current was then maintained for at least 20 s (Fig. 1*a*) and was nearly as large as in normal Ringer. Ca_o^{2+} below 3 μM usually led to a steady increase in the dark current, and higher Ca_o^{2+} to a decrease. For cones, a stable dark current was produced at a lower Ca_o^{2+} , and we usually used 0.3 μM . All responses in low- Ca^{2+} /zero- Na^+ solution have been junction corrected^{14,16}. Light intensities in photons μm^{-2} can be converted to isomerizations using a collecting area of 40 μm^2 for rods (500 nm) and about 1 μm^2 for red-sensitive cones (620 nm).



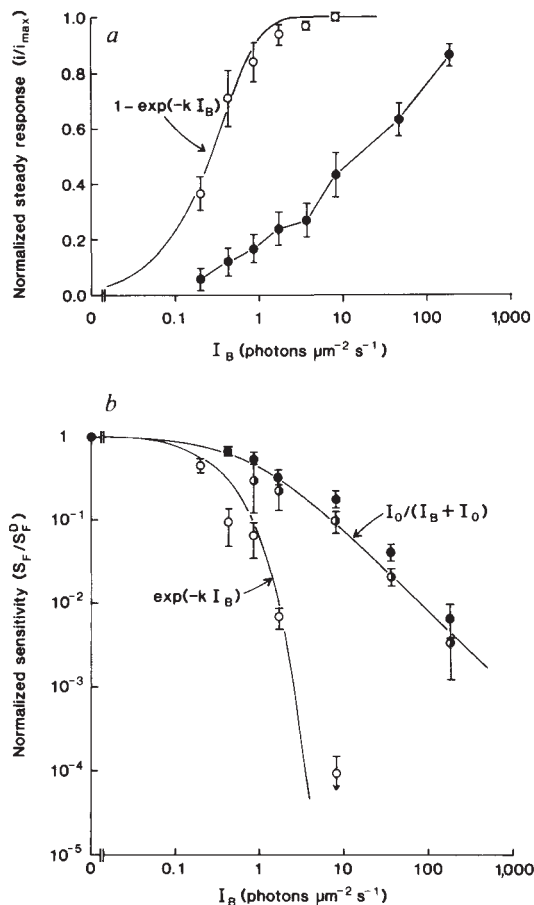


Fig. 2 Normalized steady response (a) and incremental sensitivity (b) as functions of background intensity I_B (photons $\mu m^{-2} s^{-1}$) for rods. ●, Ringer; ○, for background illumination presented during exposure to low- Ca^{2+} /zero- Na^+ ; and (in b) ●, for low- Ca^{2+} /zero- Na^+ exposures presented after equilibration to the background. Steady current response (i) has been normalized to the maximal response (i_{max}) obtained with bright light; flash sensitivity (S_F) has been normalized to the sensitivity in the appropriate solution in darkness (S_F^D). Curves are as follows. In a: for ○, $1 - \exp(-k I_B)$ with $k^{-1} = 0.38$ photons $\mu m^{-2} s^{-1}$. In b: for ○, $\exp(-k I_B)$ with the same k as in a; and for ● and ●, $I_0/(I_B + I_0)$ with $I_0 = 0.8$ photon $\mu m^{-2} s^{-1}$. Error bars represent $\pm$ s.d. Downward arrow indicates upper limit (assuming minimum detectable response of 0.5 pA) when the response was too small to be measured above the noise of the recording. Results are from seven rods in (a) and from five of the same rods in (b), but measurements were not made at each intensity for every cell. Qualitatively similar results were obtained from four additional rods for which Ca_0^{2+} was $\sim 10 \mu M$, but unbuffered.

Ringer⁶. We find that, in low- Ca^{2+} /zero- Na^+ solution, this equation fits not only steady responses as shown in Fig. 2a but also flash responses, both at fixed early times and at the peak. This suggests that, when changes in Ca_i^{2+} are prevented, rods sum responses to photons with an integration time that does not vary with the intensity or duration of illumination.

Measurements of sensitivity (Fig. 2b) show that the normal Weber-law desensitization recorded in Ringer (●)¹⁻³ was replaced by simple compressive saturation (of the form seen in primate rods²³) when the background illumination was presented during low- Ca^{2+} /zero- Na^+ exposure (○). The steep curve fitted to the open symbols in Fig. 2b is the normalized derivative, $\exp(-k I_B)$, of the curve fitted to the same symbols in Fig. 2a. Thus the light-induced decrease in incremental sensitivity in low- Ca^{2+} /zero- Na^+ solution is simply the result of the non-linearity of the response-intensity relation. The half-filled symbols in Fig. 2b give the incremental sensitivity when the low-

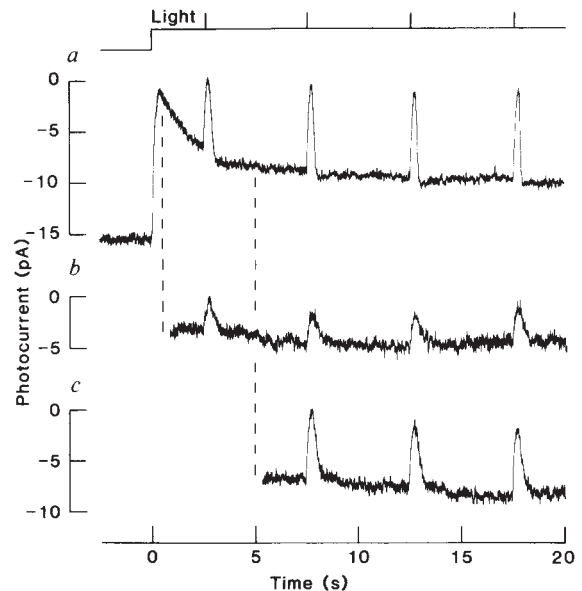


Fig. 3 Response of a red-sensitive cone to bright steps of light (1.2×10^5 photons $\mu m^{-2} s^{-1}$) presented at time zero, followed by saturating flashes (2×10^5 photons μm^{-2}). Response in (a), which was recorded with the cone in Ringer, shows that at this intensity most of the normal recovery of circulating current and response amplitude was complete within 5 s of onset of the background illumination. Stepping the outer segment to low- Ca^{2+} /zero- Na^+ solution at 0.5 s after onset of light (b) prevented normal recovery. Note the larger circulating current and responses recorded when cell was stepped to low- Ca^{2+} /zero- Na^+ solution at 5 s (c).

Ca^{2+} /zero- Na^+ exposure was presented after equilibration to the background (●). The desensitization remained in approximate agreement with Weber's law, indicating that the compressive behaviour shown by the open symbols was not the result simply of guanidinium exposure.

Another manifestation of light adaptation which is abolished in low- Ca^{2+} /zero- Na^+ solution is shown in Fig. 3 for a salamander red-sensitive cone. Upon exposure to bright illumination, the photoreceptor's response normally recovers from an initial peak to a maintained level (Fig. 3a). It is this recovery which contributes to the shallowness of the response-intensity relation to steady light¹. In Fig. 3b presentation of low- Ca^{2+} /zero- Na^+ solution at about the time of the peak in Fig. 3a prevented the normal recovery. Hence, this aspect of light adaptation also appears to be produced by a change in Ca_i^{2+} .

The experiments shown in Figs 1 and 2 (for rods) and in Fig. 3 (for a cone) have been performed on both types of photoreceptor with comparable results. Hence all of the manifestations of light adaptation in vertebrate photoreceptors seem to be abolished by treatment designed to minimize changes in Ca_i^{2+} . This statement should be qualified in two ways. First, at the high intensities needed to saturate the response of cones, photopigment bleaching becomes appreciable and makes an additional contribution to desensitization. Second, during exposures to low- Ca^{2+} /zero- Na^+ solution lasting longer than 10–20 seconds, responses to dim or moderate intensity backgrounds showed a slow recovery of circulating current. We believe that this slow recovery was caused by a change in Ca_i^{2+} , because its time course (in rods) was greatly prolonged when, in preliminary experiments, we injected the calcium buffer BAPTA (1,2-bis-(*o*-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid)²⁴ into the cytoplasm. The existence of this slow recovery indicates a gradual removal of Ca^{2+} from the cytoplasm even in the absence of Na^+ bathing the outer segment.

We conclude that Ca_i^{2+} is the messenger for light adaptation both in rods and cones, and that the requisite changes in Ca_i^{2+} are produced primarily by Ca^{2+} fluxes across the outer-segment

plasma membrane. Changes in Ca^{2+} have been shown to modulate the synthesis and hydrolysis of cyclic GMP by guanylate cyclase^{14,25-29} and phosphodiesterase^{10,30}. It will be of considerable interest to determine the relative contribution of these and any other Ca^{2+} -sensitive mechanisms to light adaptation in vertebrate photoreceptors, and to examine similar phenomena in invertebrates, where decreased sensitivity is mediated by increased Ca^{2+} (ref. 31).

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Calcium and light adaptation in retinal rods and cones

K. Nakatani & K.-W. Yau

Howard Hughes Medical Institute and Department of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA

Retinal rods and cones respond to light with a membrane hyperpolarization. This hyperpolarization is mediated by an ionic conductance (the light-regulated conductance) that is kept open in darkness by cyclic GMP acting as a ligand, and which closes in the light as a result of an increase in cGMP hydrolysis triggered by illumination¹⁻³. Calcium ions appear to have a role in this phototransduction process: they provide negative feedback between the conductance, which is permeable to Ca^{2+} (refs 4, 5), and the concentration of cGMP, which is sensitive to Ca^{2+} (refs 6-8). This feedback down-regulates the sensitivity to light of a photoreceptor and probably contributes to the important phenomenon of light adaptation in vision⁹⁻¹¹. It is still not clear, however, how much of the light adaptation is actually attributable to this Ca^{2+} feedback. We have examined the responses of amphibian rods and cones to light with the Ca^{2+} feedback removed. Normally, the response of a cell to a step of light rises transiently to a peak, but rapidly relaxes to a lower level, indicative of light adaptation. When the feedback is removed, however, the relaxation of the response is completely absent; furthermore, the steady response levels at different light-step intensities are well predicted by a statistical superposition of invariant single-photon responses. We

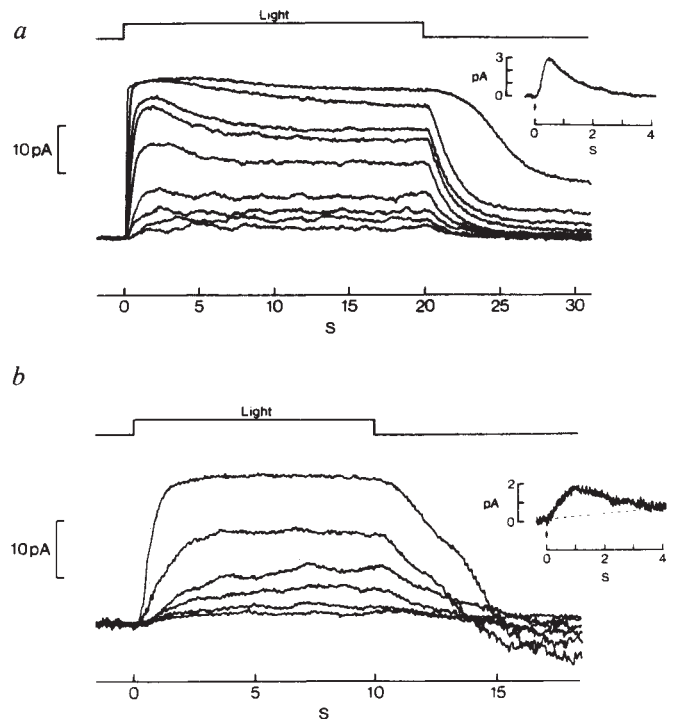


Fig. 1 Response-intensity families recorded from two rods: *a*, in normal Ringer's solution and *b*, in test solution with the Ca^{2+} feedback removed. Single trials in all traces, 520 nm light throughout. The dark current in *a* was quite stable throughout the experiment, but that in *b* showed slow variations in between light steps. To compensate for these variations in the dark current, each response amplitude plotted in Fig. 2 has been normalized against the size of dark current at the time the response was elicited. Insets: averaged responses of the cells to dim flashes in the control and the test solutions. The calculated flash sensitivity (normalized) and the response integration time, corresponding to the parameters k_f and t_i in the text, are $0.049 \text{ photon}^{-1} \mu\text{m}^2$, 1.29 s in *a* and $0.068 \text{ photon}^{-1} \mu\text{m}^2$, 2.33 s in *b*.

Methods. Isolated rods and cones from the larval tiger salamander, *Ambystoma tigrinum*, were used. These were obtained by finely chopping up a piece of dark-adapted retina under Ringer's solution and gently dispersing the chopped pieces^{11,15}. Manipulations were carried out in infrared light. Membrane current was recorded from a single cell by drawing its inner segment into a tight-fitting glass pipette containing Ringer's solution and connected to a current/voltage converter^{11,14,15}. The outer segment was subjected to bath perfusion, either with normal Ringer's solution or with the test solution. Solution switching was achieved with a valve operated pneumatically and with electronic timing, and took ~200 ms to complete¹¹. Normal Ringer's contained (in mM): 110 NaCl, 2.5 KCl, 1.6 MgCl_2 , 1.0 CaCl_2 , 5 TMA (tetramethylammonium)-HEPES, 5 dextrose, pH 7.6. The test solution contained the same ingredients except that the NaCl was replaced by guanidinium chloride and the CaCl_2 was 0-5 μM (nominal). Room temperature. All the rods and cones recorded from were of the 'red' variety, with optimal excitation wavelengths at around 520 nm and 620 nm respectively²⁴⁻²⁷. Stimulating light was unpolarized and incident approximately at right angles to the cell's longitudinal axis.

therefore conclude that the Ca^{2+} feedback underlies essentially all light adaptation in rods and cones.

In darkness, there is a circulation of Ca^{2+} at the rod or cone outer segment, consisting of an influx through the light-regulated conductance and an efflux through a Na^+ - Ca^{2+} exchange carrier^{9,11-13}. In the light, the Ca^{2+} influx is reduced or stopped but the Ca^{2+} efflux is unabated, leading to a decline in intracellular Ca^{2+} concentration^{9,11}. It is this decline in intracellular Ca^{2+} concentration that provides negative feedback to the light-activated cGMP cascade in phototransduction and makes the cGMP level resistant to decline in the light^{9,11}. We removed the Ca^{2+} feedback in a rod or cone by eliminating both the Ca^{2+} influx

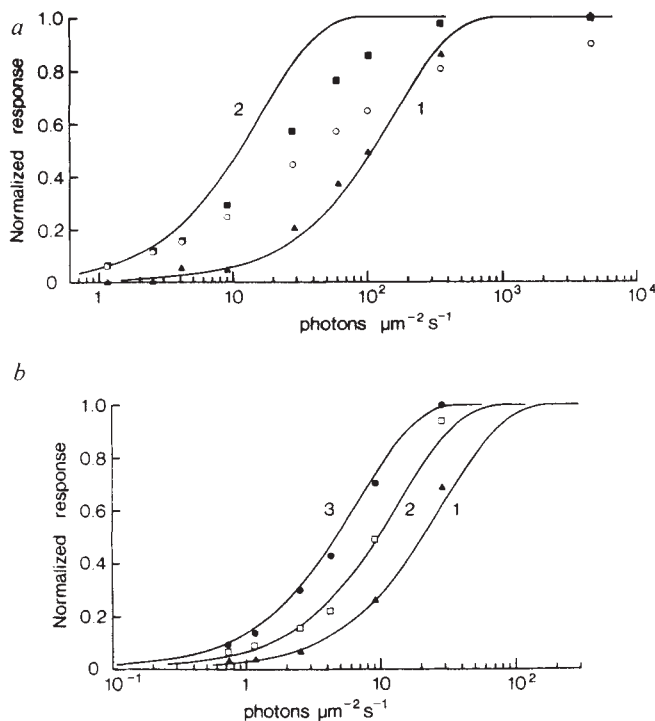


Fig. 2 Response-intensity plots obtained from the rod experiments of Fig. 1. All response amplitudes have been normalized against corresponding dark currents (see Fig. 1 legend). *a*, Response amplitudes were measured at 0.4 s after the onset of light step ($\blacktriangle$), at transient peak ($\blacksquare$) and at steady-state level just before the turning off of light ($\circ$). *b*, Response amplitudes were measured at 0.8 s ($\blacktriangle$) and 1.6 s ($\square$) after light onset, and at steady-state level ($\bullet$). The smooth curves were all drawn according to equation (1).

and efflux at the outer segment membrane. This was done by removing external Ca^{2+} and replacing external Na^{+} with the guanidinium ion, which can carry inward dark current through the light-regulated conductance but is unable to drive the Na^{+} - Ca^{2+} exchange carrier^{11,13}. Membrane current was recorded from a single salamander rod or cone with a suction pipette, and the response of the cell to light with the outer segment exposed to control or test solution was examined^{14,15}.

Figure 1*a* shows the responses of a rod in normal Ringer solution to steps of light at different intensities. Each response rises to an initial peak, then relaxes to a lower steady level within a few seconds, showing light adaptation. Figure 1*b* shows the light responses of a different rod in the test solution, which removed the Ca^{2+} feedback. This cell did not show any relaxation, suggesting the absence of light adaptation.

These results are analysed more quantitatively in Fig. 2. In *a*, the normalized response amplitudes in control Ringer are plotted against the intensities of the light steps. The response-intensity relations measured at 0.4 s after the onset of the light step, at the transient peak and at steady state just before the termination of the light are shown. The smooth curves 1 and 2 are drawn according to the relation:

$$\hat{r} = 1 - e^{-k_s I_s} \quad (1)$$

where $\hat{r}$ is the normalized response amplitude, k_s is a constant and I_s is the light step intensity (in photons $\mu\text{m}^{-2} \text{s}^{-1}$). This equation describes an overall response of the cell composed of a statistical superposition of invariant single-photon responses, each corresponding to a regional, complete closure of the light-regulated conductance^{16,17}. Thus the curves show the expected response-intensity relation if there were no light adaptation. Although the experimental data at 0.4 s fit equation (1) reasonably well, the other two sets of data progressively deviate from it, indicating the gradual development of light adaptation.

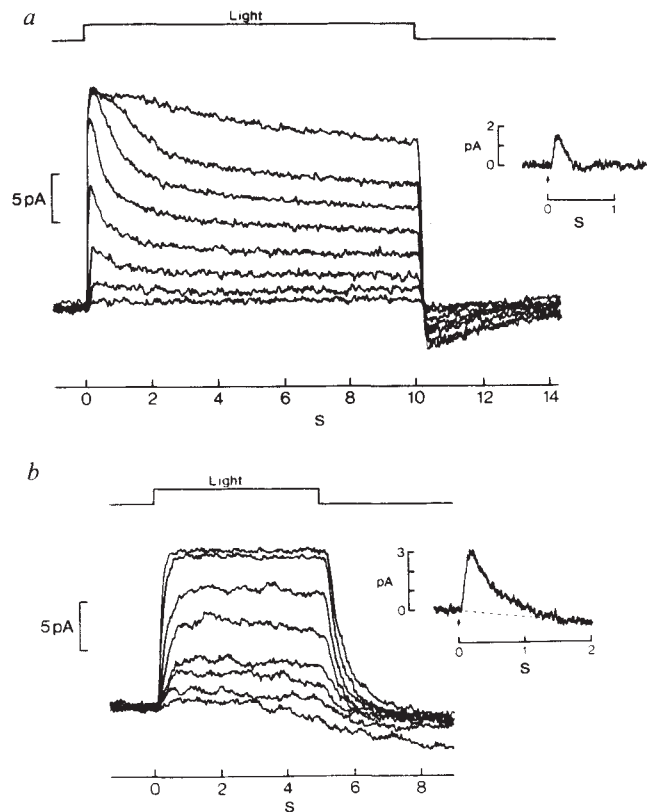


Fig. 3 Response-intensity families recorded from two cones, one in normal Ringer's solution (*a*) and the other in the test solution with the Ca^{2+} feedback removed (*b*). Single trials in all traces, 620 nm light throughout. The dark current in *a* was very stable throughout the experiment, but that in *b* showed some variations between light steps. To compensate for these variations in dark current, each response amplitude plotted in Fig. 4 has been normalized against the dark current amplitude at the time the response was elicited. Insets, averaged responses of the cones to dim flashes in the control and the test solutions. From calculations, the normalized flash sensitivity (k_f) and the response integration time (t_i) are $3.0 \times 10^{-4} \text{ photon}^{-1} \mu\text{m}^2$, 0.19 s in *a* and $5.8 \times 10^{-4} \text{ photon}^{-1} \mu\text{m}^2$, 0.45 s in *b*.

Measurements of the response amplitudes at other times (not plotted) indicate that light adaptation is obvious as early as 0.6 s after the onset of light. Curve 1 is arbitrarily positioned on the abscissa to coincide with the experimental points at low light intensities. The position of curve 2, however, which is intended to describe the steady-state situation, is constrained by the relation $k_s = k_f t_i$, where k_f is the normalized flash sensitivity of the cell (in units of $\text{photons}^{-1} \mu\text{m}^2$), obtained from its response to a dim flash in the absence of background light (inset of Fig. 1*a*), and t_i is the integration time of the response in seconds¹⁷. The dim-flash response is a scaled representation of the response of the cell to a single photon. Curve 2 fits the steady-state response amplitude at the dimmest intensity quite well, but the response rapidly falls below the curve at higher intensities, reflecting the influence of light adaptation. Similar observations were made from five other rods.

In Fig. 2*b*, the same plots were made from the experiment shown in Fig. 1*b*. The experimental points show the response-intensity relations at 0.8 s and 1.6 s after the onset of light, and at the steady-state level. The curves are again drawn according to equation (1), and here all three sets of data fit the theoretical prediction. The position of curve 3, which fits the steady-state data, has again been constrained by the amplitude and the integration time of the response of the cell to a dim flash in the absence of background light (inset of Fig. 1*b*). Such excellent agreement between experiment and theory, both in form and in

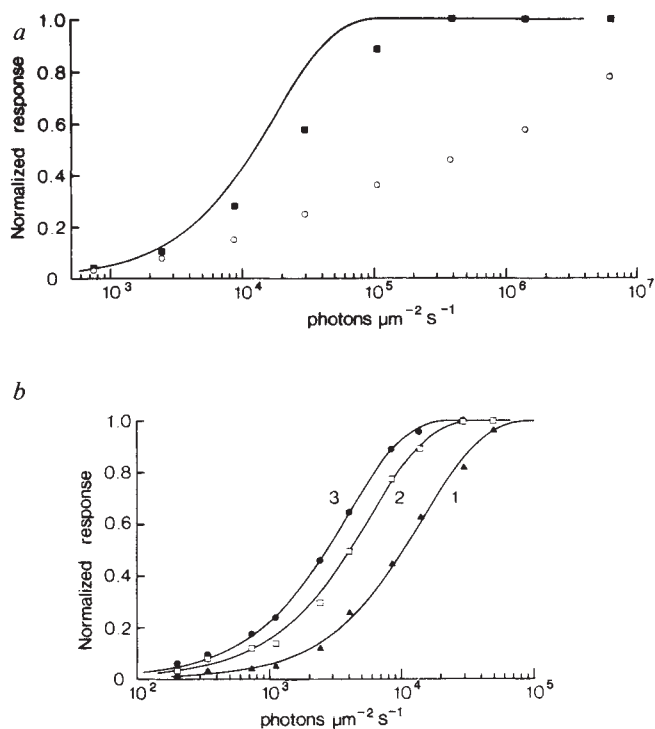


Fig. 4 Response-intensity plots obtained from the cone experiments of Fig. 3. All response amplitudes have been normalized against corresponding dark currents (see Fig. 3 legend). *a*, Response amplitudes were measured at transient peak (■) and at the end of the light step (○). *b*, Response amplitudes were measured at 0.2 s (▲) and 0.4 s (□) after light onset, and at steady-state level (●). The smooth curves were all drawn according to equation (1).

position on the light intensity axis, provides convincing evidence that the underlying single-photon effects are invariant with light intensity. Four other experiments gave similar conclusions.

Essentially the same results were obtained from cones. The response-intensity family in Fig. 3*a* was obtained from a cone in normal Ringer solution, whereas that in Fig. 3*b* was obtained from a cone in test solution. Again, the prominent relaxations shown in the control responses were absent in the test solution. Measurements made from the experiments in Fig. 3 are shown in Fig. 4. In Fig. 4*a*, the normalized response amplitudes at both the transient peak and at the end of the light step in Fig. 3*a* are plotted against light intensity; in both cases the experimental points fall substantially below the curve drawn from equation (1), indicating light adaptation. We found the same in four other experiments. In these experiments we unfortunately could not make meaningful measurements of the response amplitude at times earlier than the transient peak because the rather slow membrane time constant in cones limits the rate of rise of the response. In Fig. 4*b*, the relations between response amplitude and light intensity from Fig. 3*b* are plotted at times of 0.2 s and 0.4 s after light onset, and at the steady-state level. As for rods under the same conditions, the relations can be fitted by the theoretical relation assuming no light adaptation. Again, the position of curve 3, which fits the steady-state relation, has been calculated from the normalized amplitude and the integration time of the response of a cell to a dim flash (inset of Fig. 3*b*). The agreement between prediction and experiment is excellent, with respect to both the form of the relation and its position on the abscissa. Six other experiments gave much the same results.

The results described here strongly suggest that the Ca^{2+} feedback underlies practically all light adaptation exhibited by retinal rods and cones. As mentioned earlier, this Ca^{2+} feedback arises from a decline of free Ca^{2+} in the receptor's outer segment during illumination, which occurs roughly with a time constant

of 0.5 s in rods^{9,11} and 0.1 s in cones^{12,13}. The faster Ca^{2+} decline in cones is therefore consistent with the more rapid development of light adaptation in these cells, as shown above. The mechanism by which Ca^{2+} affects the cGMP level is still not entirely clear at present. At least part of this action appears to arise from an inhibitory effect of Ca^{2+} on guanylate cyclase, which synthesises cGMP¹⁸⁻²¹. In addition, Ca^{2+} may stimulate the cGMP phosphodiesterase, directly or indirectly^{10,22,23}. Finally, note that, although amphibian rods and cones both exhibit light adaptation, only the cones of the primate retina show this phenomenon¹⁷. A clue to the puzzle why primate rods do not light-adapt may be derived from examining the Ca^{2+} feedback in these cells.

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Picomolar concentrations of lead stimulate brain protein kinase C

Jasna Markovac & Gary W. Goldstein

Departments of Pediatrics and Neurology,
University of Michigan Medical School, Ann Arbor,
Michigan 48109-0570, USA

Recent growth studies in children suggest that there is no threshold for adverse effects from the universal exposure to inorganic lead¹. The biochemical mechanisms mediating low-level toxicity are unclear, but in several biological systems, lead alters calcium-mediated cellular processes^{2,3} and may mimic calcium in binding to regulatory proteins⁴. Here we present evidence that lead stimulates diacylglycerol-activated calcium and phospholipid-dependent protein kinase, protein kinase C, partially purified from rat brain. Picomolar concentrations of lead are equivalent to micromolar calcium in kinase activation, so this regulatory enzyme is sensitive to the lead levels expected from current environmental exposure.

Lead poisoning results in permanent brain damage⁵, high-level exposure during childhood causing distinct neurological problems and irreversible mental retardation⁶. Exposure even to subclinical levels of lead produces intelligence deficits, poor academic achievement⁷, hyperactivity, and deficient fine motor control⁸, as well as short stature and decreased weight¹. Depend-

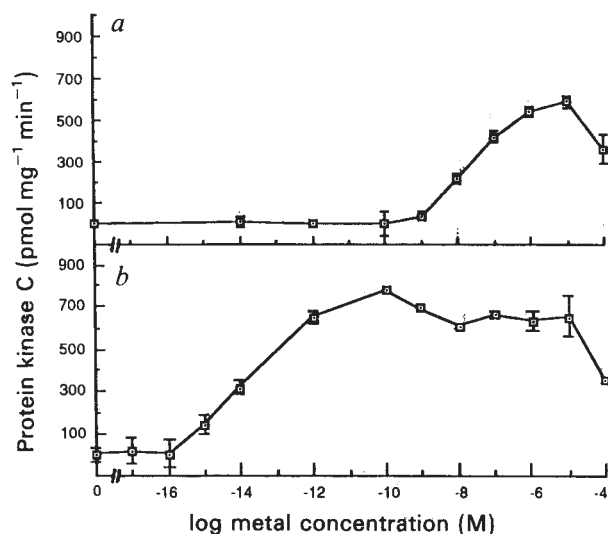


Fig. 1 Dose-dependent stimulation of protein kinase C by calcium (a) and lead (b).

Methods. Protein kinase C activity was partially purified from freshly isolated brain of adult Sprague-Dawley rats. The brains were washed and homogenized in buffer containing 20 mM HEPES, pH 7.5, 2 mM EDTA, 2 mM EGTA, 0.25 M sucrose and 10 mM 2-mercaptoethanol, and then centrifuged for 1 h at 5,000g. The resulting supernatant fraction was loaded on to a prewashed DE52 ion-exchange column. The column was washed extensively with homogenization buffer and the kinase activity was eluted with 0.2 M NaCl in the above buffer. The fractions containing protein kinase C activity were pooled and used in subsequent experiments. Protein kinase activity was assayed by a modification of the procedure described by Takai *et al.*¹³, measuring the incorporation of radiolabel from [³²P]ATP into endogenous cytosolic protein and exogenous lysine-rich histones. In 250 μ l total volume, the standard reaction mixture contained 20 mM Tris-HCl (pH 7.4), 5 mM MgCl₂, 5 μ g phosphatidylserine, 5 μ g 1,2-diolein (or equivalent volume of double distilled H₂O), 20 μ g lysine-rich histones, 0.2 nmol ATP, 2 μ Ci [³²P]ATP (4,000 Ci mmol⁻¹, ICN), and a specified concentration of a, CaCl₂ or b, lead acetate. The reaction was initiated by addition of 100 μ l pooled kinase fraction containing 1–3 μ g protein, incubated for 5 min at 30 °C and terminated by addition of 75 μ l 12M glacial acetic acid. Phosphorylated proteins were collected by adsorption on Whatman P81 phosphocellulose papers. The filters were washed once in 30% acetic acid, twice in 15% acetic acid and finally in acetone. The radioactivity was quantitated by Cerenkov counting and protein content determined by the method of Bradford²⁹. Protein kinase activity was calculated as the difference in the activity in the presence and absence of 1,2-diolein. Values plotted are the means of three replicates $\pm$ s.e.m. Data points without error bars represent s.e.m. < 5%. Equivalent results were obtained in three separate experiments.

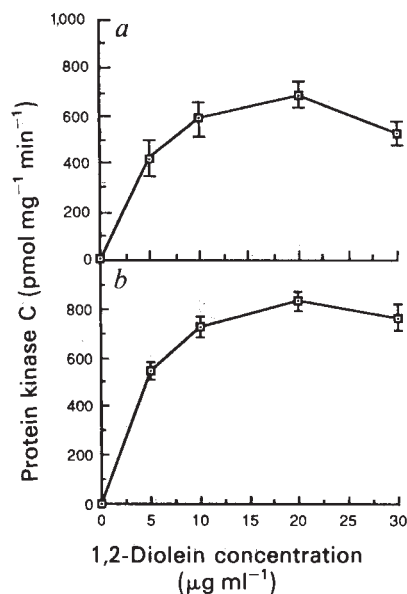


Fig. 2 Diacylglycerol-dependent activation of protein kinase C in the presence of calcium or lead. Diacylglycerol requirements of protein kinase C were tested in the presence of a, 10⁻⁵ M calcium, or b, 10⁻¹⁰ M lead. CaCl₂ or lead acetate at the appropriate concentration was added to the standard reaction mixture and the kinase activity assayed in the presence of various amounts of 1,2-diolein (0–30 μ g ml⁻¹). Values plotted are the means of three replicates $\pm$ s.e.m. Data points without error bars represent s.e.m. < 5%. Equivalent results were obtained in three separate experiments.

ing on the criteria used to define excessive exposure, as many as 9–25% of pre-school children are at risk for adverse effects from low levels of lead⁹. The biochemical mechanisms of lead toxicity are unknown, but at least some of its deleterious effects are attributed to interference with calcium-mediated processes^{2,3}, and lead is known to alter the metabolism of calcium in several tissues¹⁰.

Protein kinase C is a calcium- and phospholipid-dependent enzyme that can mediate cellular proliferation, differentiation and function by phosphorylating critical regulatory proteins¹¹. This kinase is activated by a diacylglycerol second messenger produced by receptor-mediated hydrolysis of inositol phospholipids. Inositol triphosphate is another product of this hydrolysis that causes the release of calcium from the endoplasmic reticulum¹². We chose to investigate the effect of lead on protein kinase C because it is regulated by calcium¹³ and is critical in the control of cellular signal transduction¹¹.

Protein kinase C was partially purified from fresh rat brain (see Fig. 1 legend for methods and assay conditions). Enzyme activity was recovered by salt elution using ion exchange chromatography and measured as diacylglycerol-specific incorporation of radiolabel from [³²P]ATP into exogenous lysine-rich histones in the presence of lead or calcium. Figure 1 illustrates the stimulation of protein kinase C as a function of calcium (Fig. 1a) and lead (Fig. 1b) concentration. There was no detectable diacylglycerol- and phospholipid-dependent protein kinase activity in the absence of either cation. Kinase activity increased with increasing calcium concentration, with a threshold in the nanomolar range, peaked at 10⁻⁵ M, and then declined. This decrease in activity reflects a reduced affinity of protein kinase C for diacylglycerol at higher calcium concentrations. A similar decline was observed in the presence of lead at concentrations >10⁻⁵ M, suggesting an analogous mechanism for interaction with protein kinase C. Protein kinase activity was stimulated by much lower concentrations of lead, with a threshold in the picomolar range and a peak at 10⁻¹⁰ M. We compared the requirement for diacylglycerol of the kinase activity stimulated by lead at 10⁻¹⁰ M with the calcium-stimulated enzyme. Using varying amounts of 1,2-diolein (0–30 μ g ml⁻¹), the kinetic plots generated from these experiments were quite similar in general shape (Fig. 2). There was no detectable phospholipid-dependent protein kinase activity with either cation in the absence of diolein. Comparison of the time course for protein kinase C activation by either lead or calcium also indicates a similar degree of protein kinase C-mediated substrate phosphorylation by 10⁻¹⁰ M lead and by 10⁻⁵ M calcium (data not shown). In the presence of either cation, the reaction was linear up to 10 min incubation at 30 °C. To determine whether the picomolar threshold for the activation of protein kinase C is specific to lead or is merely a ubiquitous effect of heavy metals, we tested several different cations and heavy metals for their ability to stimulate the kinase activity. As shown in Table 1, only lead was able to activate this enzyme at 10⁻¹⁰ M to a degree comparable with

Table 1 Activation of protein kinase C by heavy metals

Condition	Protein kinase C activity (pmol mg ⁻¹ min ⁻¹)
No addition	11.8 ± 45.3
Pb	550.1 ± 77.3
Ca	43.8 ± 70.8
Hg	17.9 ± 44.1
Cu	22.4 ± 40.9
Zn	47.3 ± 10.2
Ba	34.9 ± 14.3
Mb	38.8 ± 16.4
Sr	33.9 ± 6.7
Co	9.5 ± 20.4
Fe	11.6 ± 57.7
Mn	37.2 ± 33.0
Ni	8.4 ± 54.9

Each metal was tested in the standard reaction mixture at a concentration of 10^{-10} M as a substitute for CaCl_2 in the activation of protein kinase C. Values represent means of three determinations $\pm$ s.e.m. Lead showed statistically significant activation of protein kinase C at $P < 0.002$ using a one-tailed *t*-test with Bonferroni correction for multiple comparisons²⁸. All other metals tested were not significantly different from the untreated control at $P \leq 0.05$.

micromolar calcium. Different lead salts at picomolar concentrations were also assayed for ability to activate the enzyme to verify that the lead cation itself was responsible for the stimulation of protein kinase C. The lead salts were all found to be equally effective, and sodium acetate gave us no detectable diacylglycerol-activated, phospholipid-dependent protein phosphorylation (see Table 2).

Protein kinase C phosphorylates various critical cell membrane and transport proteins¹¹, and is thus a major site for regulation of cellular growth and differentiation. The enzyme is thought to be the cellular receptor for tumour-promoting phorbol esters¹⁴. Mapping of these receptors and studies with monoclonal antibodies indicate that protein kinase C is widely distributed in the brain^{15,16}. Using highly immunospecific polyclonal antibodies, the enzyme in the brain has been localized largely to presynaptic terminals¹⁷. This finding is consistent with an important role for protein kinase C-mediated protein phosphorylation in the regulation of presynaptic function¹⁷. Lead accumulates in the synaptic regions of the brain and is thought to interfere with the release of neurotransmitters^{18,19}. Here we present evidence that picomolar concentrations of lead activate partially purified rat brain protein kinase C to an extent similar to that stimulated by micromolar calcium. At 10^{-10} M lead, the requirement of the protein kinase for diolein was similar to that found with 10^{-5} M calcium, the standard condition for assay of protein kinase C. This suggests that lead mimics calcium in the activation of protein kinase C.

Nishizuka and coworkers investigated the effects of several heavy metals on this enzyme and found that at micromolar concentrations some can activate protein kinase C²⁰, but lead was not included in these investigations. We find that the response at picomolar levels was specific for lead (Table 1). Several studies report other lead-induced biochemical effects. At micromolar concentrations, lead can reversibly inhibit $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ activity²¹. At concentrations $>10^{-5}$ M lead interferes with acetylcholine metabolism, a calcium-dependent process²². Over the same concentration range, this toxicant also inhibits the calcium-mediated α -adrenergic regulation of pyruvate kinase activity²³. Earlier work has shown that micromolar lead activates calmodulin-sensitive phosphodiesterase and promotes potassium loss from erythrocytes by replacing calcium²⁴. We investigated the effects of lead on calcium/calmodulin-dependent protein kinase activity and found a stimulatory effect at 10^{-5} M but no activation at 10^{-10} M (data not shown). The activation of protein kinase C by picomolar levels of lead is

Table 2 Effect of lead salts and sodium acetate on protein kinase activity

Salt	Protein kinase C activity (pmol mg ⁻¹ min ⁻¹)
None	ND*
Lead acetate	610.1 ± 47.2
Lead chloride	672.0 ± 64.6
Lead citrate	635.7 ± 78.8
Sodium acetate	ND

Protein kinase C was activated by the addition of the specified compound to the standard reaction mixture at 10^{-10} M. Values represent means of three determinations $\pm$ s.e.m. All three lead salts showed statistically significant activation of protein kinase C at $P < 0.005$ using a one-tailed *t*-test with Bonferroni correction for multiple comparisons.

* No detectable protein kinase C activity.

particularly significant considering the concentrations found in patients exposed to the toxin. Lead has no known biological value, so its optimal concentration in living systems is zero. Because of worldwide environmental exposure, the 'normal' blood concentration of lead is between 5 and 25 μg per 100 ml of whole blood or 10^{-6} M (ref. 9). Relatively small increases in the blood concentration of lead (>30 μg per 100 ml) are toxic²⁵, and variations within the accepted normal range may produce adverse neurobehavioural^{8,26} and growth effects¹. As 95–98% of the lead is bound in red blood cells, $\sim 10^{-8}$ M is present in the plasma²⁷. If the distribution of lead between plasma and cytosol is analogous to that of calcium (10,000:1), the cytosolic concentration of lead in exposed individuals should be in the picomolar range. Therefore if a specific enzyme is a target for lead toxicity, then it must be sensitive to extremely low concentrations. To our knowledge, the stimulation of protein kinase C represents the first observation of a lead-induced biochemical alteration in the picomolar range. As a result of its effects on protein kinase C, lead can potentially induce changes in both the specificity and rate of substrate phosphorylation by this enzyme. We propose that the marked sensitivity of protein kinase C to lead makes this regulatory enzyme a potential mediator of lead toxicity.

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Force measurements by micromanipulation of a single actin filament by glass needles

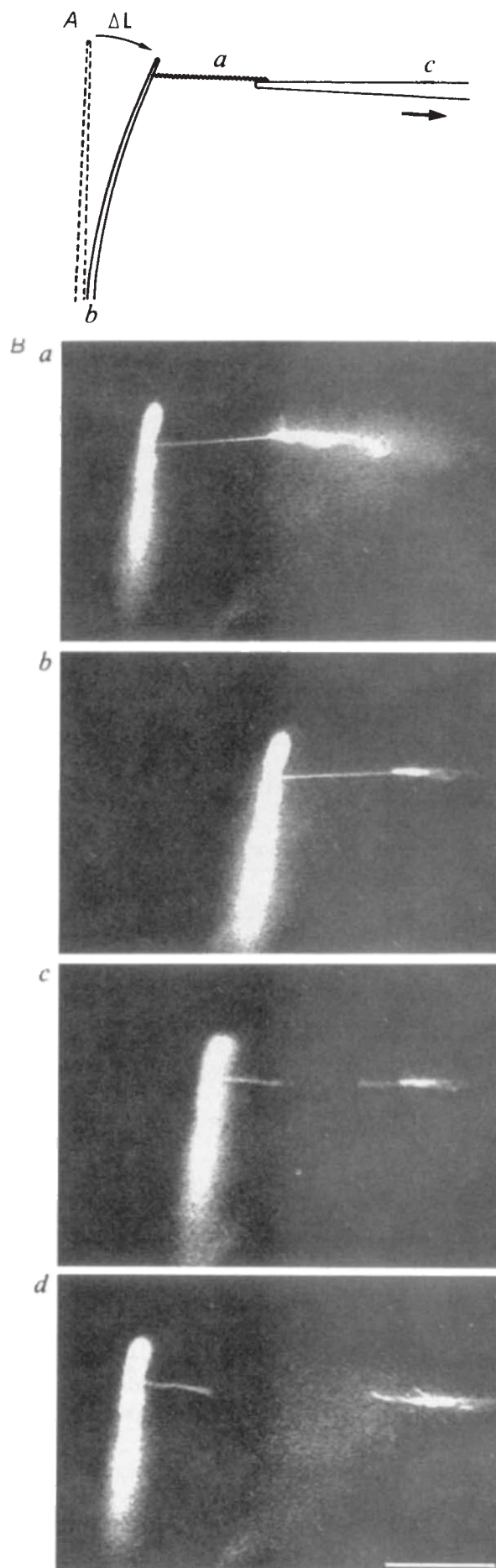
Akiyoshi Kishino and Toshio Yanagida

Department of Biophysical Engineering,
Faculty of Engineering Science, Osaka University, Toyonaka,
Osaka, Japan

Single actin filaments (~ 7 nm in diameter) labelled with fluorescent phalloidin can be clearly seen by video-fluorescence microscopy¹. This technique has been used to observe motions of single filaments in solution and in several *in vitro* movement assays¹⁻⁵. In a further development of the technique, we report here a method to catch and manipulate a single actin filament (F-actin) by glass microneedles under conditions in which external force on the filament can be applied and measured. Using this method, we directly measured the tensile strength of a filament (the force necessary to break the bond between two actin monomers) and the force required for a filament to be moved by myosin or its proteolytic fragment bound to a glass surface in the presence of ATP. The first result shows that the tensile strength of the F-actin-phalloidin complex is comparable with the average force exerted on a single thin filament in muscle fibres during isometric contraction. This force is increased only slightly by tropomyosin. The second measurement shows that the myosin head (subfragment-1) can produce the same ATP-dependent force as intact myosin. The magnitude of this force is comparable with that produced by each head of myosin in muscle during isometric contraction.

Actin filaments labelled with phalloidin-tetramethylrhodamine, which stabilizes the filament structure of actin⁶, were dispersed in solution. Both ends of a single actin filament were caught using two kinds of microneedles connected to micromanipulators under a fluorescence microscope. One of the needles, used for measuring force, was very flexible, and the other, used for pulling actin filaments, was stiff. Before the experiments, the needles were coated with monomeric myosin to increase their affinity for actin. Force was measured according to the method of Kamimura and Takahashi, who used the bending of calibrated microneedles to measure the force of microtubules sliding in flagella⁷. In the present study, the tensile strength of an actin filament was obtained by pulling the stiff needle until the filament broke, and measuring the maximum bending of the flexible needle just before the filament broke (Fig. 1*a,b*). The elastic coefficient of the flexible needles (the force required to move the end of the needle by $1\ \mu\text{m}$; see Fig. 1), is $1.5\text{--}10\ \text{pN}\ \mu\text{m}^{-1}$.

Figure 2 shows the tensile strength of single F-actin filaments as a function of length from 4 to $32\ \mu\text{m}$. The tensile strength of F-actin is 108 ± 5 (s.d., $n=61$) pN and almost independent of the filament length. This value is hardly changed by adding 10 mM phosphate. This is the first direct measurement of the actin-actin bond strength in F-actin (with phalloidin). This tensile strength is nearly ten times smaller than the force necessary to break the actin-actin bond in F-actin estimated from a potential profile of the monomer-monomer interaction in F-actin, which was theoretically predicted from the elastic modulus for bending and the equilibrium free energy of actin-actin bond in F-actin⁸. Binding of tropomyosin to F-actin increased the tensile strength only slightly (117 ± 7 pN, s.d., $n=22$), suggesting that the bond strength between tropomyosin molecules in the thin filament is weak. This result is consistent with modelling⁹ and crystallography¹⁰ studies of tropomyosin, in which the linkage between molecules appears to be a weak point contact of the head of one molecule with the tail of another.



The tensile strength of F-actin or the F-actin-tropomyosin complex is comparable to (slightly less than) the average force exerted on a single thin filament (F-actin-tropomyosin-troponin complex) in muscle during the development of isometric tension (120–200 pN)⁸. This result is consistent with the observation that long actin filaments are easily shortened to small fragments during movement along myosin bound to a glass surface^{2–5}. This small value of tensile strength may arise from the effect of phalloidin or of strong illumination. Because phalloidin stabilizes the filament structure of F-actin⁶ and does not affect the mechanical properties of muscle¹¹, it is unlikely that phalloidin weakens F-actin. The effect of incident light is dramatically decreased by removal of oxygen from solution. In oxygen-free solution, an actin filament can be held for longer than 10 min under illumination by light about five times more intense than that used for our earlier experiments, and can be stretched by a force only slightly less than that required for breaking it (~100 pN).

It is also unlikely that temperature was increased significantly by illumination, because the velocity of actin filaments along myosin bound to glass surface is unchanged when the light intensity is varied from five times higher to 30 times lower than that used for tensile strength experiments, although this filament velocity is very sensitive to the temperature ($Q_{10} = 2$). Therefore, we expected the effect of illumination to be negligibly small. Thus, our results indicate that the tensile strengths obtained here are unexpectedly small but are intrinsic properties of F-actin and F-actin-tropomyosin. Because the actin filament is stretched with both ends rigidly bound to the needles, a force different from that involved in muscle contraction, for example, strong torsional strain, might be exerted on it.

Myosin, a large molecule of relative molecular mass 480,000, consists of two globular heads, containing catalytic and actin-

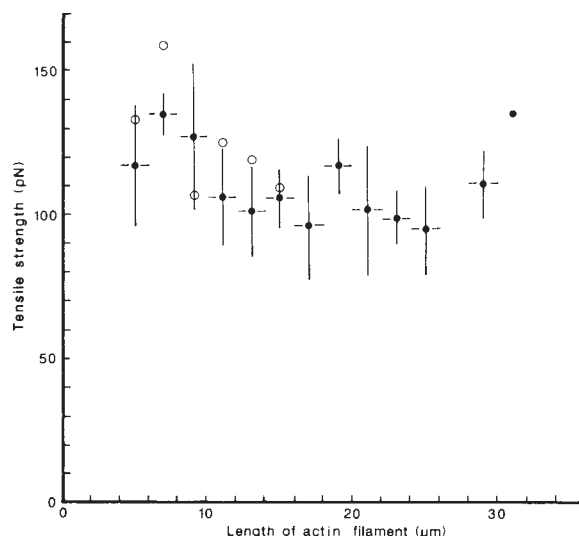


Fig. 2 Relationship between tensile strengths and lengths of F-actin and F-actin-tropomyosin complex. ●, F-actin. Bars indicate s.d. ($n = 3-10$); ○, F-actin-tropomyosin complex. Each point indicates mean for 2–5 measurements.

◀ Fig. 1 A, The principle of measuring tensile strength of F-actin; B, sequential fluorescence micrographs of a single F-actin filament, labelled with fluorescent phalloidin, being caught and stretched by the microneedles. A, One end of a single F-actin filament (a) floating in solution was first bound to a flexible needle (b) and the other end was bound to a stiff needle (c). F-actin was stretched by moving the stiff needle to the right. The tensile strength, was obtained from the displacement (ΔL) of the flexible needle required to break the F-actin filament. B, Both ends of F-actin are attached to the two microneedles before stretching (a). The stiff needle (right) is moved to the right, pulling the F-actin and bending the flexible needle (b). This image was recorded just before the filament broke. c, After the F-actin was broken; d, the flexible needle returns to the original position. The elastic coefficient of the flexible needle was $4.0 \text{ pN } \mu\text{m}^{-1}$. Because the illumination is not homogeneous, the brightness of objects is stronger around the centre of the picture than near the edges. We easily confirmed that the caught filament is a single actin filament by comparing its image with those of single filaments floating in solution. The needles are bright because many fluorescent F-actin filaments stick to the surface of the needles. Bar, $20 \mu\text{m}$.

Methods. Microneedles mounted on micromanipulators (Narishige) were treated with silicone and coated with monomeric myosin. The elastic coefficient of flexible needles was determined as follows¹⁸. The compliance of the stiff needle was measured by a semiconductor strain gauge and then the compliance of the second needle, nearly ten-fold smaller, was determined by cross-calibration with it. The compliance of a third, more flexible, needle was determined by cross-calibration with the second one. By repeating this cycle 5–7 times, the compliance of flexible needles used for the experiments was determined. Ten measurements were performed for each cycle and the s.d. was within 5% of the mean. F-actin was labelled with phalloidin-tetramethyl-rhodamine¹ and suspended in solution (50 mM KCl, 20 mM HEPES pH 7.0, 2.5 mM MgCl_2 and 1% 2-mercaptoethanol) at an actin-monomer concentration of 25 nM. Before experiments, 0.1 mg glucose-oxidase ml^{-1} ; 0.018 mg catalase ml^{-1} ; and 2.3 mg glucose ml^{-1} were added to the solutions to remove oxygen (see text). Fluorescent F-actin filaments were observed and manipulated under an inverted microscope (Nikon) equipped with epifluorescence optics, a Zeiss Neofluar $\times 100$ objective (oil immersion, NA, 1.3), and a 100-W mercury arc lamp. Fluorescent images were recorded on videotape with a high-sensitivity television camera (Ikegami SIT camera CTC-9000) and a video recorder (Victor CR 6650). All proteins were obtained from rabbit skeletal muscle. Actin, tropomyosin and myosin were purified by standard methods¹. S-1 was prepared by papain digestion of myosin¹⁹ and purified by $(\text{NH}_4)_2\text{SO}_4$ fractionation and gel filtration (Ultragel AcA 34). All experiments performed at $25 \pm 2^\circ\text{C}$.

binding sites, and a coiled-coil rod, which forms the core of thick filament. The region of the molecule essential for transduction of chemical energy to mechanical energy has been studied by *in vitro* movement assays^{4,5,12–14}. In a recent report it was clearly shown that the head portion (subfragment-1 or S-1) is sufficient to produce movement of actin⁵. However, in those assays, because the actin filaments are unconstrained, negligible force is required to move them. Therefore, it has not until now been known whether S-1 can produce significant force, and this remains a crucial question for elucidation of the molecular mechanism of muscle contraction.

Therefore, we directly measured the force generated by intact myosin and S-1 bound to a glass surface coated with silicon (Sigmacote, Sigma). One end of a single actin filament was bound to a flexible needle coated with *N*-ethylmaleimide (NEM)-treated myosin so that actin filaments were rigidly bound to the needle in the presence of ATP. Another end was placed in contact with a myosin- or S-1-coated glass surface in the presence of ATP. The actin filament moves on the glass surface and pulls the needle, bending it to a stable position (Fig. 3).

The force was measured from the displacement of the flexible needle, using the elastic coefficient of the needle ($5-10 \text{ pN } \mu\text{m}^{-1}$). Figure 4 shows the relationship between the measured force and the length of actin filaments in contact with the surface. From the slopes of the least-squares lines in Fig. 4, the average force per micrometre of actin filament touching the surface, coated with monomeric myosin and S-1, is 9.6 and 5.4 pN, respectively. The densities of myosin and S-1 on the surface were obtained by measuring the Ca^{2+} -ATPase activity of myosin and S-1 on the surface, respectively⁵. The ATPase activity was measured by a high-sensitivity assay for Pi using malachite green¹⁵. The densities of heads of myosin and S-1 were $3.2 \times 10^{11} \text{ cm}^{-2}$ and $1 \times 10^{11} \text{ cm}^{-2}$, which correspond to average nearest-neighbour distances of 19 and 34 nm, respectively.

These results lead to the conclusion that myosin and S-1 exert a comparable force per head calculated simply from the number density of heads bound to the coverslip. Taking into account the fact that myosin heads or S-1 bound to the surface can interact only with one of the two strands of the F-actin helix at the same position, we can calculate the maximum numbers of myosin heads and S-1 that can interact with a $1 \mu\text{m}$ F-actin filament to be $(1 \text{ head per } 19 \text{ nm}) \times 1 \mu\text{m} = 53$ heads for myosin and $(1 \text{ head per } 34 \text{ nm}) \times 1 \mu\text{m} = 30$ heads for S-1. Therefore,

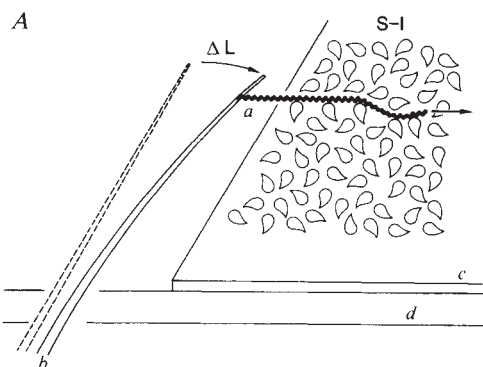
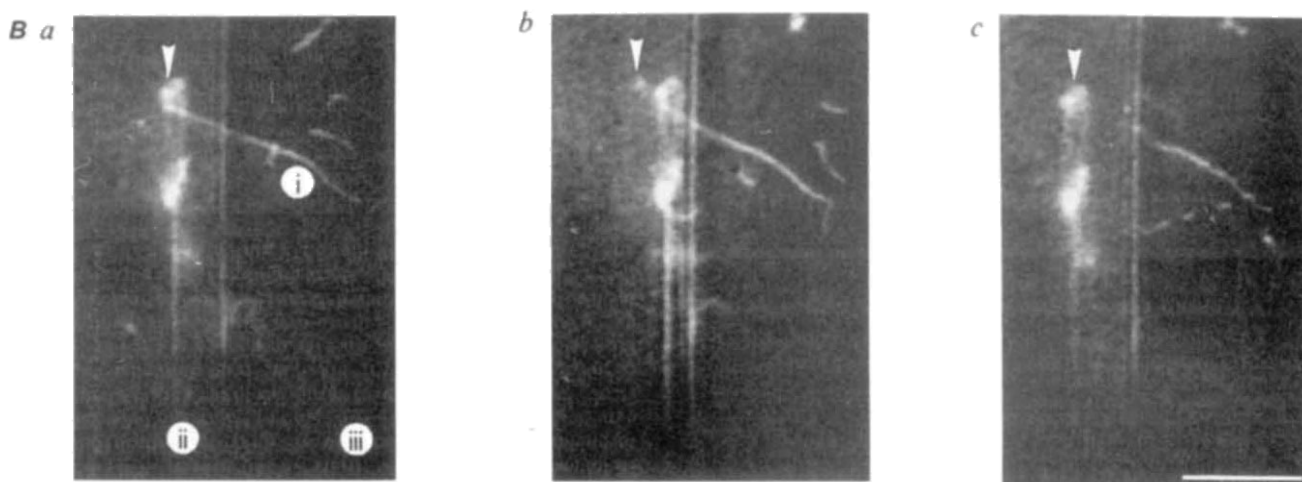


Fig. 3 A, The principle of measurement of force produced by S-1 (or myosin) bound to a glass surface. B, Sequential fluorescence micrographs of a single F-actin with one end fixed on a flexible needle and the other end lying on an S-1-coated surface in the presence of MgATP. A, a thin glass plate (c), with about a 10- μ m thick surface coating of silicone, was placed on a coverslip (d) and coated with S-1 or monomeric myosin. The silicone treatment allows us to observe reproducible ATP-dependent movement of F-actin on S-1 and monomeric myosin bound to the surface (Y. Harada and T.Y., in preparation). A single fluorescently labelled F-actin filament (a) with one end fixed on a flexible needle (b), which had been previously coated with NEM-treated myosin, was touched on a myosin- or S-1-coated surface (c) in the presence of ATP. In about half of the cases, F-actin pulled and bent the needle, and in the other cases, actin moved in the opposite direction. a, F-actin; b, flexible needle; c, S-1-coated thin glass plate; d, coverslip. B, The flexible microneedle is about to be pulled (a) and the force produced by the interaction between F-actin and S-1 is balanced by the bending force of the needle (b). (c), The F-actin is detached from the needle and the needle returns to its original position. Solutions: 35 mM KCl, 20 mM HEPES pH 7.8, 2.5 mM $MgCl_2$, 1 mM ATP and 1% 2-mercaptoethanol. Before the experiments, oxygen was removed from the solution as described in Fig. 1. i, F-actin; ii, flexible needle; iii, S-1-coated glass surface. Arrowheads, position of the flexible needle under zero force. Bar, 10 μ m.



the minimum force per head if all heads within range of an actin could exert force is about 0.2 pN (9.6 pN per 53 heads for myosin and 5.4 pN per 30 heads for S-1). Because the orientations of myosin and S-1 on the surface are random and therefore all heads are not expected to be able to interact with the actin, this result is an underestimate and the force per head is probably two or three times larger and therefore comparable to force

exerted in muscle during isometric contraction (about 1 pN)⁸.

By combining the present technique with the recently developed techniques for measuring nanometric displacement under an optical microscope^{16,17}, we hope to study in more detail the strength of the monomer-monomer bond in F-actin and to follow directly the process of force generation by a single myosin head coupled to the ATP hydrolysis reaction. These experiments are in progress.

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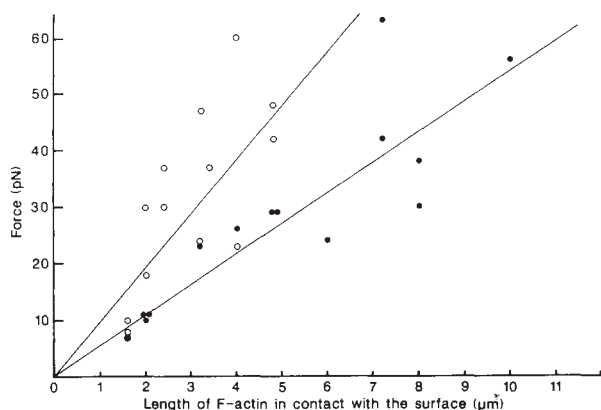


Fig. 4 Relationship between the force and the length of actin filament that is in contact with the surface coated by either monomeric myosin (○) or S-1 (●). Because the actin filaments are usually not at the right angle to the needle (Fig. 3B), the force generated was obtained as: bending force of needle $\times \csc \theta$, where θ is the angle between the needle and F-actin filament.

A yeast activity can substitute for the HeLa cell TATA box factor

Bruno Cavallini*, Janine Huet†, Jean-Luc Plassat*,
André Sentenac†, Jean-Marc Egly* & Pierre Chambon*‡

* Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS, Unité 184 de Biologie Moléculaire et de Génie Génétique de l'INSERM, Faculté de Médecine, 11 rue Humann, 67085 Strasbourg Cédex, France

† Département de Biologie, Service de Biochimie, Centre d'Etudes Nucléaires de Saclay, 91191 Gif-Sur-Yvette Cédex, France

Most class B (II) promoter regions from higher eukaryotes contain the TATA box and upstream and enhancer elements¹. Both the upstream and enhancer elements and their cognate factors have regulatory functions, whereas the TATA sequence interacts with the TATA box factor BTF1 to position RNA polymerase B and its ancillary initiation factors (STF, BTF2 and BTF3) to direct the initiation of transcription ~30 base pairs downstream². In many respects, class B promoter regions from the unicellular eukaryote *Saccharomyces cerevisiae* are similarly organized, containing upstream activating sequences that bear many similarities to enhancers^{3,4}. Although they are essential for initiation, the yeast TATA sequences are located at variable distances and further from the start sites (40–120 base pairs), whose locations are primarily determined by an initiator element⁴. The basic molecular mechanisms that control initiation of transcription are known to be conserved from yeast to man: the yeast transcriptional *trans*-activator GAL4 can activate a minimal TATA box-containing promoter in human HeLa cells, and a human inducible enhancer factor, the oestrogen receptor, can activate a similar minimal promoter in yeast^{5–8}. This striking evolutionary conservation prompted us to look for the presence in yeast of an activity that could possibly substitute for the human TATA box factor. We report here the existence of such an activity in yeast extracts.

The possible presence of a yeast factor that may substitute for the HeLa cell BTF1 TATA-box factor was investigated by complementation of a HeLa transcription system defective in BTF1 with chromatographic fractions derived from yeast extracts. The activity of yeast fractions was followed by monitoring transcription of a template containing the Adenovirus-2 major late promoter (Ad2MLP), truncated upstream of the TATA box at position -34 (pM34, see ref. 9). Using a HeLa cell transcription system containing RNA polymerase B, STF, BTF2 and BTF3 factors² and the HEPO.4 fraction as a source of purified BTF1 factor (see legend to Fig. 1), the Ad2MLP template pM34/*Sal*I (ref. 9) yields a 309 nucleotide-long RNA run-off transcript (Fig. 1, lane 3). Addition of crude yeast extracts in place of the HeLa BTF1 factor yielded no specific transcripts (data not shown). Accordingly, an S100 cytoplasmic extract¹⁰ was applied to a heparin-Ultrogel column and the 0.6M KCl eluate fraction was separated into various fractions by stepwise elution from a DEAE-5PW HPLC column as previously described^{2,11}. Addition of the 0.08M KCl fraction (DEY fraction) to an incubation mixture containing RNA polymerase B and the STF, BTF2 and BTF3 factors, but lacking the BTF1 factor, yielded an Ad2MLP 309-nucleotide-long RNA run-off transcript identical to that obtained with the HeLa BTF1 factor (Fig. 1, lanes 1–3). Similar results were obtained using a different Ad2MLP template (pM34/*Ava*II, see legend to Fig. 2) which yielded a 457-nucleotide-long run-off transcript (data not shown, see also Figs 2 and 3). Thus, an activity present in the DEY yeast fraction can substitute for the HeLa BTF1 factor. The DEY fraction was also indispensable for efficient transcription of other class B TATA box-containing promoters (see legend

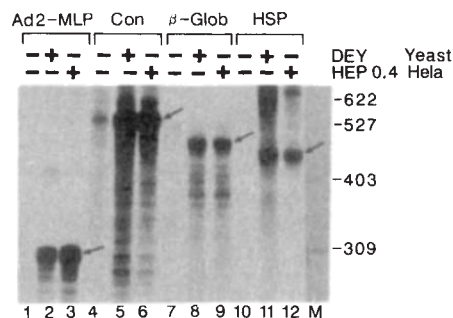


Fig. 1 Transcription of various RNA polymerase B promoters. The DNA fragments encompassing the various promoters were assayed for transcription activity in 25 μ l reactions containing 50 mM Tris-HCl, pH 7.9, 6 mM MgCl₂, 0.1 mM EDTA, 0.5 mM dithiothreitol (DTT), 50 mM KCl, 8.7% glycerol and 0.1% NP40 detergent, in the presence of HeLa cell RNA polymerase B (0.002 units), the three other indispensable general transcription factors [STF (0.9 μ g DEO.35 fraction), BTF2 and BTF3 (3 μ g DEO.15 fraction)¹¹], and either the HeLa HEPO.4 fraction (0.3 μ g) (see below) or the yeast DEY fraction (0.5 μ g), as indicated at the top of the figure. After a preincubation period of 15 min at 24 °C, nucleoside triphosphates were added to start RNA synthesis. Incubation was continued at 24 °C for 45 min, then the reaction was stopped and the transcripts analysed on polyacrylamide/urea gels¹². Template DNA, lanes 1–3: Ad2MLP, 60 ng *Eco*RI-*Sal*I fragment of pM34 (ref. 9), run-off transcript 309 nucleotides; lanes 4–6: chicken conalbumin promoter (Con), 60 ng *Eco*RI-*Bam*HI fragment of plasmid pCON-37 containing the conalbumin sequences from -272 to +203 inserted between pBR322 positions 1 and 29, run-off transcript 549 nucleotides; lanes 7–9, rabbit β -globin, 225 ng *Pst*I-*Bam*HI fragment of pG2 plasmid²⁴, run-off transcript 475 nucleotides; lanes 10–12, *Drosophila* Hsp70, 200 ng *Taq*I-digested pHSPox plasmid, a derivative of plasmid 132E3 (ref. 25), run-off transcript 455 nucleotides. Arrows point to the specific run-off transcripts. Lane M, *Msp*I-digested pBR322 plasmid. Autoradiography exposure time was 3 days.

Methods. Preparation of the yeast DEY fraction. An extract of yeast *S. cerevisiae* S100 (protein concentration 37 mg ml⁻¹; 35 ml) prepared as described elsewhere¹⁰ was dialysed overnight against buffer A (50 mM Tris-HCl, pH 7.9, 100 mM KCl, 5 mM MgCl₂, 0.5 mM DTT, 17.4% glycerol, 0.1 mM PMSF) and loaded on to a 150 ml heparin-Ultrogel column (IBF, France) equilibrated in buffer A. The column was then washed sequentially with buffer A (200 ml); buffer A containing 0.24 M KCl (250 ml) and finally 0.6 M KCl (200 ml). The HEP 0.6 M fraction was diluted to 0.5 M KCl and applied to a 400 ml DEAE-Cellulose (Whatman DE22) column equilibrated in buffer A containing 0.5 M KCl to eliminate the nucleic acids. The flow-through fraction was dialysed overnight against buffer B (50 mM Tris-HCl, pH 7.9, KCl 20 mM, 5 mM MgCl₂, 0.1 mM EDTA, 0.5 mM DTT, 8.7% glycerol, 0.1 mM PMSF), applied to a preparative HPLC DEAE 5PW column (2.15 $\times$ 15 cm) (Toyo Soda) and eluted with a discontinuous KCl gradient. The TATA box factor-like activity was present in the 0.08 M eluted fraction (DEY).

Preparation of the HeLa HEPO.4 fraction. The HeLa cell DE 0.25 fraction (see ref. 2 for the purification scheme) containing the BTF1 factor was applied to an SP-5PW ion exchanger (Toyo Soda) and the SP 0.28 M KCl peak fraction chromatographed on a Blue Trisacryl column (IBF-France). The 0.5 M KCl eluted fraction containing the BTF1 activity was then loaded onto a heparin HPLC column (Toyo Soda). The activity was recovered in the 0.4 M KCl fraction.

to Fig. 1): chicken conalbumin (Con, lanes 5 and 6), rabbit β -globin (β -Glob, lanes 8 and 9) or *Drosophila* heat shock 70 (HSP, lanes 11 and 12). The same run-off transcripts were obtained in all cases with either the HeLa TATA box factor or the yeast DEY fraction, and S1 nuclease mapping data (not shown) indicated that the same start sites were used with either factor.

To test the possibility that the DEY fraction could contain a TATA box-specific DNA binding factor, we used a competition assay described previously¹². Briefly, a competing TATA box-

‡ To whom correspondence should be addressed.

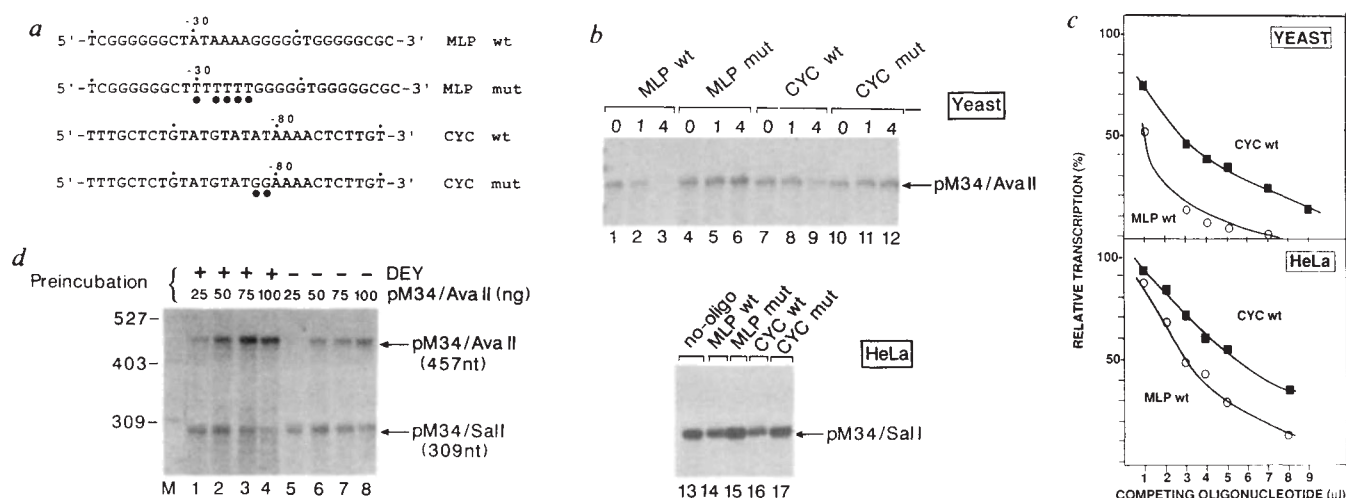


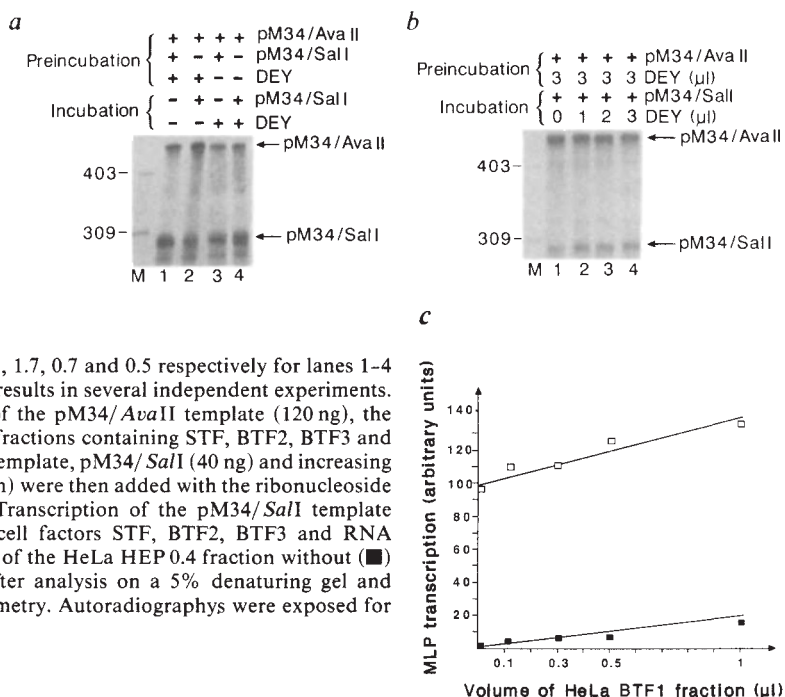
Fig. 2 The yeast factor interacts with TATA sequences and participates in the formation of a stable preinitiation complex. **a**, Oligonucleotides used in the competition assays: Ad2MLP (MLP wt) and mutant (MLP mut); *S. cerevisiae* isocytochrome *c*, wild type (CYC wt) and mutant (CYC mut). Mutated bases (closed circles) were selected for their deleterious effect on transcription *in vivo*. Numbering above the sequences indicates the position relative to the first main *in vivo* messenger RNA startsite affected by the TATA box mutation. **b**, Competition assays, lanes 1–12: increasing amounts (1 and 4 μ l as indicated; 0.15 pmol per μ l) of the different oligonucleotides were preincubated with the yeast DEY fraction (0.5 μ g) for 15 min at 24 °C before addition of the complementary fractions containing STF, BTF2 and BTF3, RNA polymerase B (as described in the legend to Fig. 1) and the pM34/AvaII DNA template (60 ng); lanes 13–17: the same assay was performed with the HeLa HEP0.4 fraction (0.1 μ g) containing the BTF1 factor and 4 μ l the various oligonucleotides. After preincubation, the nucleoside triphosphates were added to start RNA synthesis as described in the legend to Fig. 1. **c**, Densitometer quantitation of the data shown in panel **b** and of data (not shown) obtained in several competition experiments which gave similar results ($\pm 10\%$). Upper panel, yeast DEY fraction; lower panel: HeLa BTF1 factor. Symbols —■— and —○—, CYC wt and MLP wt competing oligonucleotides respectively. No significant competition was observed with the corresponding mutant oligonucleotides (data not shown). **d**, Different amounts (25–100 ng, as indicated) of the first DNA template (the AvaII/AvaII fragment of pM34, pM34/AvaII) were preincubated for 15 min at 24 °C with (lanes 1–4) or without (lanes 5–8) the DEY fraction (0.5 μ g), as indicated at the top of the figure. A fixed amount (30 ng) of the second DNA template (pM34/SalI) was then added with the other complementary fractions containing STF, BTF₂, BTF₃, RNA polymerase B (see legend to Fig. 1) and DEY (0.5 μ g) for lanes 5–8. After further preincubation for 5 min and addition of the ribonucleotides, transcription was continued for 45 min (see legend to Fig. 1). Autoradiography exposure time was 24 h.

containing oligonucleotide was preincubated with the DEY fraction before addition of the pM34/AvaII template, RNA polymerase B and the other HeLa cell transcription factors (except BTF1) required for *in vitro* transcription. Competing double-stranded oligonucleotides contain either the wild-type Ad2MLP TATA box (MLP wt, see Fig. 2a) or the wild-type *S. cerevisiae* isocytochrome *c* TATA box (CYC wt) sequences. Preincubation with increasing amounts of TATA box-containing oligonucleotides (see Fig. 2b, lanes 1–3 and lanes 7–9 for MLP wt and CYC wt respectively, and densitometered data in Fig. 2c) clearly decreased transcription from the Ad2MLP pM34/AvaII template subsequently added. In contrast, no decrease in transcription was observed when the DEY fraction was preincubated with the oligonucleotide sequences containing either the Ad2MLP (MLP mut) or isocytochrome *c* (CYC mut) TATA boxes that bore mutations known to decrease the transcription from the corresponding promoters *in vivo* (refs 9 and 13 respectively) (see panel b, lanes 4–6 and 10–12). As a control, parallel assays were performed with the HEP0.4 HeLa cell fraction containing the BTF1 factor. As expected from our previous results, preincubation with the wild-type TATA box oligonucleotides, but not with the mutated sequences, resulted in a decrease of transcription from the Ad2MLP promoter (Fig. 2b, compare lanes 13–17). In agreement with these results, no specific run-off transcriptions were observed when we used an Ad2MLP template containing a mutated TATA box (pM4X, see ref. 9; data not shown). Autoradiogram densitometer scans like those in Fig. 2b show that when the MLP wt oligonucleotide was used as a competitor, the level of transcription was reduced to an almost undetectable level in the presence of either the yeast DEY or the HeLa BTF1 fraction (panel c). The fact that the TATA box-containing CYC wt competitor was apparently less efficient than MLP wt in both cases probably reflects a lower

affinity of both TATA box factors for the CYC than for the MLP TATA sequence. In this respect, note that roughly 2–3-fold increased amount of CYC wt oligonucleotide was required to achieve a 50% competition, irrespective of the origin of the TATA box factor. The larger amount of competing oligonucleotides that is required to achieve the same inhibition when using the HeLa extract instead of the DEY fraction could reflect differences in TATA box factor concentrations in the human and yeast fractions.

To address the question as to whether the yeast activity present in the DEY fraction can substitute for the HeLa BTF1 factor for the formation of a stable preinitiation complex, the DEY fraction was preincubated with increasing amounts of the pM34/AvaII template in the absence of the other transcription components. Subsequently, a second template pM34/SalI was added together with these components and transcription measured from both templates using the run-off assay (Fig. 2d, lanes 1–4). In a parallel set of assays (lanes 5–8) the DEY fraction was left out of the preincubation mixture. Preincubation of increasing amounts of pM34/AvaII template with the DEY fraction resulted not only in an increase in transcription from this template (457-nucleotide RNA run-off), but also in a concomitant decrease in the RNA corresponding to the second template pM34/SalI (309 nucleotide RNA run-off) (lanes 1–4). But when the DEY fraction was added together with the second template after the preincubation period, the amount of each RNA transcript was consistent with the relative template concentrations (lanes 5–8). It appears, therefore, that the yeast DEY activity can form a preinitiation complex by binding sufficiently tightly to the first template to lead to its preferential transcription. In contrast to its stimulatory effect on the preinitiation complex formed by HeLa BTF1 factor^{12,14,15}, however, the addition of HeLa STF factor (DE 0.35 fraction) to the preincubation mixture

Fig. 3 The DEY fraction does not operate through activation of the HeLa TATA box factor. *a*, The DEY fraction (0.5 μ g) was incubated for 15 min at 24 °C with the pM34/*Ava*II template (80 ng) in the presence or absence of the second DNA template pM34/*Sal*I (60 ng) (as indicated at the top of the figure), together with the other HeLa cell complementary fractions containing STF, BTF₂, BTF₃ and RNA polymerase B as described in the legend to Fig. 1. If they were not included in the preincubation step, the DEY and/or the pM34/*Sal*I template (60 ng) were added with the ribonucleoside triphosphates to start the RNA synthesis. The ratios of the pM34/*Ava*II template transcript to that of the pM34/*Sal*I template were 0.85, 1.7, 0.7 and 0.5 respectively for lanes 1–4 by densitometry of the autoradiogram. We obtained similar results in several independent experiments. *b*, Preincubation was for 15 min at 24 °C in the presence of the pM34/*Ava*II template (120 ng), the yeast DEY fraction (3 μ l) and all the other complementary fractions containing STF, BTF₂, BTF₃ and RNA polymerase B (see legend to Fig. 1). The second DNA template, pM34/*Sal*I (40 ng) and increasing amounts (as indicated) of DEY fraction (0.16 μ g μ l⁻¹ protein) were then added with the ribonucleoside triphosphates and transcription continued for 45 min. *c*, Transcription of the pM34/*Sal*I template (60 ng) in the presence of all the complementary HeLa cell factors STF, BTF₂, BTF₃ and RNA polymerase B (see legend to Fig. 1) and increasing amounts of the HeLa HEP 0.4 fraction without (■) or with (□) a fixed amount of DEY fraction (0.5 μ g). After analysis on a 5% denaturing gel and autoradiography, the transcripts were quantified by densitometry. Autoradiographs were exposed for 24 h for panels *a* and *b*.



has no effect on transcription of the preincubated template present (data not shown).

Two types of experiments were performed to rule out the possibility that the DEY fraction contains an activity that may activate traces of BTF1 factor, possibly contaminating the various partially purified HeLa fractions used here. Firstly, all the HeLa cell transcription factor-containing fractions (except BTF1) were preincubated either with the pM34/*Ava*II template alone (Fig. 3*a*, lanes 2 and 4, in which cases the pM34/*Sal*I template was added after the preincubation period) or with both pM34/*Ava*II and pM34/*Sal*I templates (lanes 1 and 3) in the presence (lanes 1 and 2) or absence (lanes 3 and 4, in which case it was added after the preincubation period) of the DEY fraction. Clearly, preferential transcription of the pM34/*Ava*II template preincubated in the absence of the pM34/*Sal*I template occurred only when the DEY fraction was present in the preincubation mixture (compare lanes 2 and 4, for which the densitometer scans of the bands yielded a pM34/*Ava*II to pM34/*Sal*I ratio of 1.7 in lane 2, whereas this ratio was 0.5 in lane 4), indicating that the various HeLa fractions used here are not contaminated by significant amounts of BTF1 factor. In contrast, and as expected, the relative transcription from the two templates added together was very similar, irrespective of the presence or absence of the DEY fraction in the preincubation mixture (compare lanes 1 and 3 where pM34/*Ava*II to pM34/*Sal*I ratios were 0.85 and 0.70 respectively, and see legend to Fig. 3*a*). We also determined the minimal amount of the first template pM34/*Ava*II necessary to saturate the TATA box factor present in a preincubation mixture containing the STF, BTF₂ and BTF₃ factors, RNA polymerase and the DEY fraction; the second template pM34/*Sal*I was then added together with increasing amounts of DEY fraction (Fig. 3*b*). Under these conditions, no increase of RNA transcript from the first template was observed (densitometer scanning indicated that the intensity of the pM34/*Ava*II bands in lanes 2, 3 and 4 relative to that in lane 1 was 1.1, 1.0 and 1.0 respectively), whereas transcription from the second template increased with increasing amounts of the DEY fraction (the relative intensities of the pM34/*Sal*I bands were 1, 1.35, 1.50 and 1.65 in lanes 1, 2, 3 and 4 respectively). As a pM34/*Sal*I transcript could be detected in lane 1, then not all of the DEY TATA factor was titrated during the preincubation period (compare with lanes 1 and 2 in Fig. 1, where no transcription could be detected in the absence of added

DEY fractions). These results further support our conclusion that the DEY fraction contains an activity that can substitute for that of the HeLa cell TATA box factor. Moreover, when increasing amounts of HeLa BTF1 factor were added to incubation mixtures containing a fixed amount of the DEY fraction and an Ad2MLP template in excess (Fig. 3*c*), the stimulatory effects of the two fractions were clearly additive and not synergistic, excluding the possibility that the stimulatory activity of the DEY fraction could be due to the activation of a 'latent' form of the HeLa TATA box factor. Finally, it should be noted that the DEY fraction had no effect on nonspecific transcription by RNA polymerase B (data not shown).

Our present data strongly suggest that yeast contains an activity that can replace that of the human TATA box factor. The yeast activity, similarly to the mammalian TATA box factor, interacts preferentially with wild-type TATA box sequences from lower or higher eukaryotes to form a stable preinitiation complex. The partially purified yeast activity can complement a HeLa cell transcription system which lacks the BTF1 TATA factor for the initiation of efficient transcription from a variety of TATA-box containing promoters, irrespective of the presence or absence of upstream element(s). It has been shown that initiation of transcription *in vitro* requires the addition of initiation factors BTF₂ and BTF₃ and RNA polymerase B to the preinitiation complex^{2,12,16}. If the assembly of this initiation complex operates through protein-protein interactions between the TATA box factor and RNA polymerase B and/or the other initiation factors, then our present results indicate that these interactions have been conserved during evolution from yeast to man. In this respect we note that the largest subunit of RNA polymerase B is well conserved between yeast, *Drosophila*, mouse, calf and human from immunological¹⁷ and sequence^{18–20} data. Moreover, some upstream element regulatory factors postulated to interact with the TATA box factor^{21,22} are conserved between yeast and man²³. From the results presented in the paper of Metzger *et al.*⁷ and other recent reports^{5–8}, we can postulate that some features of the TATA box factor must be conserved between yeast and man to allow it to interact with both other factor(s) of the basic transcription apparatus and/or RNA polymerase B, and *trans*-acting factors binding to upstream and enhancer elements. Our results support this possibility and suggest that heterologous yeast-human transcription systems may be useful in determining the nature of

these interactions.

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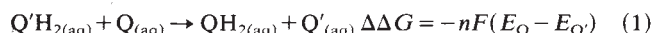
Computed redox potentials and the design of bioreductive agents

Christopher A. Reynolds, Paul M. King
& W. Graham Richards

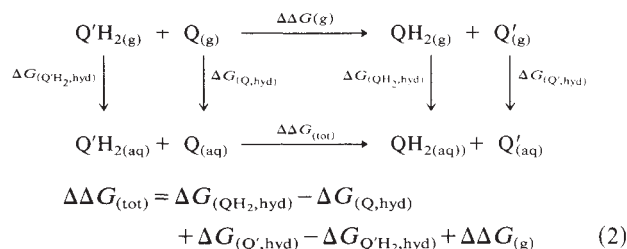
Physical Chemistry Laboratory, South Parks Road,
Oxford OX1 3QZ, UK

Anti-cancer agents that have been made selective for tumour cells by exploiting the known differences in the availability of oxygen between normal and transformed cells are a promising development in cancer chemotherapy¹. We have recently suggested a new type of bioreductive activity which would depend on a two-electron reduction^{2–4}. For rational design of such compounds, it is essential to be able to predict the redox potentials and the chemical modifications needed to produce the optimum redox value. Calculating redox potentials is a daunting task for the theoretician, however, as the effect of water solvation is clearly of major significance. Recent successful calculations^{5–10} of differences in the free energies of biologically important molecules in aqueous solution using the free-energy perturbation method prompted us to apply the technique to the computation of two-electron redox potentials. The results are accurate to within 20 mV, suggesting that we should be able to manipulate redox potentials by successfully predicting structures with the appropriate value.

Quinones are one class of molecules which have been used as bioreductive agents¹¹. The difference in redox potentials of two quinones, Q and Q', can be obtained from the free energy for the equation



This in turn can be obtained from the following thermodynamic cycle



where $\Delta G_{(QH_2,hyd)} - \Delta G_{(Q,hyd)}$, the difference in free energies of hydration of QH₂ and Q, can be obtained using the free-energy perturbation relationship

$$\Delta G = -RT \ln \langle \exp(-H_{AB}/RT) \rangle_A \quad (3)$$

H_{AB} is the difference in the Hamiltonian of states A and B, and $\langle \rangle_A$ indicates that an average is to be taken over the reference state A. This relationship is due to Zwanzig¹² and has since been implemented and applied within the framework of molecular dynamics and Monte Carlo simulations by Postma¹³, McCammon *et al.*^{6,14,15}, Jorgensen¹⁶, and by Kollman *et al.*^{5,7–10}. We have used the implementation of the method, within the framework of molecular dynamics, incorporated in the AMBER suite of programmes¹⁷. Using molecular dynamics, it is possible to sample the important regions of configurational space of the system and so evaluate equation (3) as a time average. The hamiltonian is formulated within a molecular mechanics framework (that is, the interaction potential is calculated as a sum of bond, angle, torsional, non-bonded and electrostatic terms). In general, however, if the difference in the hamiltonian of states A and B (in this case the hydrated hydroquinone and quinone) is large, equation (3) would converge very slowly. We have therefore evaluated the change by a series of simulations (windows). The hamiltonian of the system (and hence the set of molecular mechanics parameters) is defined in terms of a coupling parameter, λ according to the equation

$$H_\lambda = \lambda H(QH_2) + (1 - \lambda) H(Q) \quad (4)$$

where $\lambda = 1$ for the hydroquinone and $\lambda = 0$ for the quinone. The coupling parameter takes a value of 1 in the first simulation and is then incremented by -0.05 for 20 successive windows. In this way, atoms which appear in both initial and final solutes are gradually transformed, while those that are unique to a solute are slowly created or annihilated. To ensure that the average is evaluated over the most important configurations, the system was equilibrated for each window, before data collection. The total free energy is then the sum of the free energies obtained for each window. The results are shown in Table 1.

The term H_{AB} in equation (3), as implemented, includes only the intermolecular non-bonded and electrostatic components of the energy. This means that the change in the free energy of the solute A as it is changed to B is not evaluated. The differences in the gas-phase free energies, $\Delta\Delta G_{(g)}$, have therefore been calculated using *ab initio* molecular orbital theory within the Hartree-Fock (HF) framework¹⁸ and using Møller-Plesset second-order perturbation theory (MP2)¹⁹—see Table 2. Zero-point, thermal and entropy corrections have been obtained from harmonic frequencies calculated using the AM1 semi-empirical molecular orbital method²⁰. These results are shown in Table 3; the overall results are shown in Table 4.

While there have been other studies relating redox potentials to theoretical calculations, although without the explicit inclusion of water molecules, the present approach is very illuminating as it indicates the size of the hydration contribution. This is comparable with the gas-phase contribution and is acting in opposite directions in the two quinone systems. The closest agreement with experiment²¹ is obtained using the *ab initio*

Table 1 Differences in free energy of hydration of oxidized and reduced quinones

	$\Delta G_{(Q,hyd)} - \Delta G_{(QH_2,hyd)}$ 1,4-hydrobenzoquinone → 1,4-benzoquinone (kJ mol ⁻¹)	$\Delta G_{(Q',hyd)} - \Delta G_{(Q'H_2,hyd)}$ 1,2-hydrobenzoquinone → 1,2-benzoquinone (kJ mol ⁻¹)	$\Delta\Delta G_{(hyd)}$ (kJ mol ⁻¹)
Simulation 1			
Forward	19.53	-6.23	25.75
Backward	20.06	-7.96	28.02
Average	19.79	-7.09	26.89
Simulation 2			
Forward	25.79	-4.79	30.58
Backward	23.55	-9.33	32.88
Average	24.67	-7.06	31.73
Average (1 & 2)	22.23 (±2.44)	-7.08 (±0.02)	29.31 (±2.46)

The table also shows the hydration component of $\Delta\Delta G$ for reaction (1). The molecular dynamics simulation was carried out using ~570 TIP3P water molecules²² at constant temperature and pressure (1 atmosphere, 298K), using SHAKE²³ to constrain bonds to allow a molecular dynamics step length of 0.002 ps. Initially, equilibration was carried out for 5.6 ps. At each window, 500 steps of equilibration were followed by 500 steps of data collection. (In simulation 2, the 500 steps of equilibration were followed by 50 steps of data collection. The average and standard deviation of the two simulations is also shown. The results from simulation 1 are considered to be more accurate, however.) The parameters of the solutes were generated in a manner consistent with the AMBER program²⁴⁻²⁶.

Table 2 *Ab initio* energies/hartrees

Method Basis set for: Energy geometry	1,4-benzoquinone		1,2-benzoquinone		$\Delta\Delta H_{(ai)}$
	Oxidized	Reduced	Oxidized	Reduced	
HF/STO-3G//HF/STO-3G	-374.35335	-375.57292	-374.35026	-375.57489	13.27
HF/3-21G//HF/3-21G	-377.10068	-378.29789	-377.08913	-378.30364	45.41
HF/6-31G**//HF/3-21G	-379.23372	-380.40734	-379.21921	-380.41082	47.25
HF/6-31G**//HF/6-31G*	-379.23557	-380.40950	-379.22136	-380.41295	46.39
HF/6-31G**//HF/6-31G*	-379.24089	-380.43007	-379.22842	-380.43353	41.83
MP2/6-31G**//HF/3-21G	-380.32174	-380.51846	-380.31085	-381.52265	39.60
MP2/6-31G**//HF/6-31G*	-380.31886	-381.51862	-380.30747	-381.52247	40.03

These calculations used the GAUSSIAN 82 and CADPAC programs^{27,28} (1 Hartree = 2,625.5 kJ mol⁻¹). These energies represent absolute energies at 0K without the zero-point correction. The enthalpy at 298 K may be obtained by adding the zero-point energy and thermal enthalpy data given in Table 3. The free energy at 298 K may also be obtained using the entropy given in Table 3. The calculations used the STO-3G, 3-21G, 6-31G* and 6-31G** basis sets^{29,30}. MP2/6-31G**//HF/3-21G denotes that the energy was obtained using Møller-Plesset second order perturbation theory with a 6-31G* basis set at a geometry obtained using a Hartree-Fock calculation with a 3-21G basis set. $\Delta\Delta H_{(ai)}$ represents the gas phase enthalpy per kJ per mol for reaction (1) at 0K without the zero-point correction.

Table 3 AM1²⁰ contributions to the heat of formation, zero-point energy, thermal enthalpy and entropy (as $-T\Delta S$)

Contribution (kJ mol ⁻¹)	1,4-benzoquinone		1,2-benzoquinone		$\Delta\Delta X$
	Oxidized	Reduced	Oxidized	Reduced	
Heat of formation	-104.918	-274.788	-94.742	-277.512	12.900
Zero-point	233.325	292.185	233.141	292.252	-0.251
Thermal enthalpy	18.784	20.626	18.968	20.778	0.032
Entropy ($-T\Delta S$)	-98.258	-99.725	-99.296	-101.798	1.03

(The AM1 heat of formation includes the zero-point and thermal contributions) $\Delta\Delta X$ represents the change in energy, or entropy (for each contribution) for reaction (1).

values calculated using the 3-21G basis set; the calculated and experimental values differ by only 8 mV. The most accurate level of theory used—MP2—gives $\Delta E = -0.072$ V—a discrepancy of only 20 mV compared with experiment. All the *ab initio* calculations (except with the STO-3G basis set) give good agreement, but the semi-empirical AM1 method does not give very reliable results in this case.

The remarkably good agreement clearly results from the cancellation of errors and is not fortuitous. It is a consequence of the free-energy perturbation method and also arises when taking differences in *ab initio* energies of similar molecules. While it is difficult to estimate the errors in *ab initio* calculations and the errors due to the parameters used for the simulation, Singh has suggested that the statistical errors in the simulations may

Table 4 Free energy and redox potential differences between 1,2-benzoquinone and 1,4-benzoquinone

Component	Free energy difference (kJ mol ⁻¹)	Standard redox potential difference (V)
$\Delta\Delta H_{(ai)}$	40.031	-0.207
Thermal enthalpy	0.032	-0.000
Entropy ($-T\Delta S$)	1.034	-0.005
Zero-point	-0.251	0.001
Hydration	-26.886	0.139
Total (theory)	13.959	-0.072
Experiment		-0.092

The individual contributions to the free energy are shown in bold in Tables 1-3. The total free energy, $\Delta\Delta G_{(tot)}$ is obtained by adding the zero-point, thermal enthalpy, entropy and hydration terms to the appropriate *ab initio* value of $\Delta\Delta H_{(ai)}$. The experimental value is an average taken from ref. 21.

be estimated from the standard deviation of results from independent simulations⁷. We ran a second simulation which suggests an accuracy of 13 mV for the two simulations. The discrepancy between calculated and experimental values is of this order.

The calculations we have described were performed on the Rutherford Appleton Laboratory CRAY X-MP and on a Convex

C-1 XP2 system and make serious computational demands. We are therefore adapting the method to use transputers so that the resources needed will not be beyond the scope of typical research laboratories. The ability to compute redox potentials should permit the rational design of bioreductive agents with a specified difference in free energy between oxidized and reduced forms. Furthermore, it now appears possible to calculate redox potentials of systems for which experimental determination is not possible.

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The three-dimensional structure of a DNA duplex containing looped-out bases

Leemor Joshua-Tor*, Dov Rabinovich*, Håkon Hope†, Felix Frolow*, Ettore Appella‡, Joel L. Sussman*§

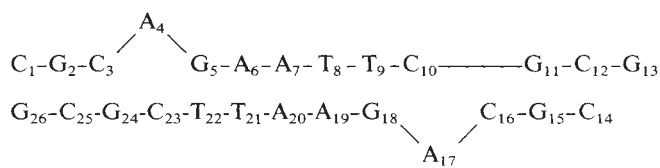
* Department of Structural Chemistry, The Weizmann Institute of Science, Rehovot 76100, Israel

‡ Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health Bethesda, Maryland 20205, USA

Unpaired bases in DNA have been assigned a possible role in the mechanism of frameshift mutagenesis in sequences with repeated base pairs¹. They also occur in quasipalindromic DNA sequences, which have been implicated in mutagenesis where there are no repeated base pairs, through the formation of single-stranded hairpin loops^{2,3}. The conformation of unpaired bases in DNA has been the subject of numerous thermodynamic as well as high resolution NMR (nuclear magnetic resonance) studies (reviewed in ref. 4). The NMR studies in solution⁵ have shown that the duplex of the tridecamer DNA fragment d(CGCAGAATTCGCG) remains intact, and that the unpaired adenosines are stacked into the duplex. Having crystallized this oligonucleotide and determined its structure, we find its conformation in the crystal is close to that of a B-DNA duplex, with the two additional adenosines looped out from the double helix and causing little disruption of the rest of the structure.

The introduction of an addition base in an otherwise fully complementary double helix could, in principle, produce several alternative structures as shown schematically in Fig. 1 for the tridecamer. These include either a misalignment of the bases in the vicinity of the additional base (Fig. 1a), or accommodation of the extra base without drastic effect on the other base pairs by a looping-out of the extra base away from the duplex (Fig. 1b), or its insertion into the double helix (Fig. 1c). A two-dimensional NMR study⁶ of a very similar sequence d(CGC-AGAGCTCGCG) which differs only in the two middle base pairs, strongly suggests that the additional adenosines in both sequences are stacked into the duplex.

Details of the synthesis by the phosphotriester solid-phase method, purification and preliminary crystallization conditions of the duplex:



have been reported⁷. Shock-cooling to $\sim -150^\circ\text{C}$ (ref. 8) resulted in virtually no radiation-induced damage and thus extended the lifetime of the crystals in the X-ray beam almost indefinitely. X-ray data were collected on a Rigaku AFC5-R rotating anode diffractometer operated at 15 kW. The space group is C2 with unit-cell dimensions $a = 78.48$, $b = 42.84$, $c = 25.16$, $\beta = 99.36$, and one DNA duplex per asymmetric unit. We observed no significant changes in the unit-cell dimensions when the crystal was cooled from 4 to -150°C and virtually no changes during data collection. The structure was solved using ULTIMA⁹ and refined to an R -factor of 15% and a correlation coefficient of 0.95.

Single-crystal X-ray analysis reveals a strikingly different conformation for the unpaired adenosines of the tridecamer in the solid state, as compared to the one derived from solution NMR studies. The overall structure is close to that of B-DNA¹⁰ but the two additional adenosines loop out from the duplex and are not inserted as observed in solution. This looping out is accomplished with minimal disruption of the overall conformation of the helix (Fig. 2) with a mean helix rotation (for the 12 base pairs) of 35° and rise per base pair of $3.38 \text{ \AA}$, as calculated by the program HELIX (written by John Rosenberg)^{11,12}. Figure 3 shows a difference Fourier map in which the atoms belonging to one of the extra adenosines (A17) were omitted from the calculated structure factors. The difference map for the other extra adenosine (A4) is of similar quality. The two adenosines

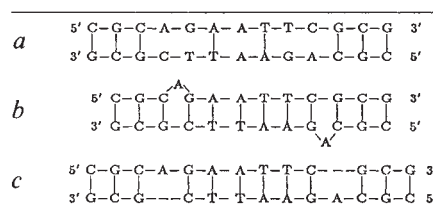
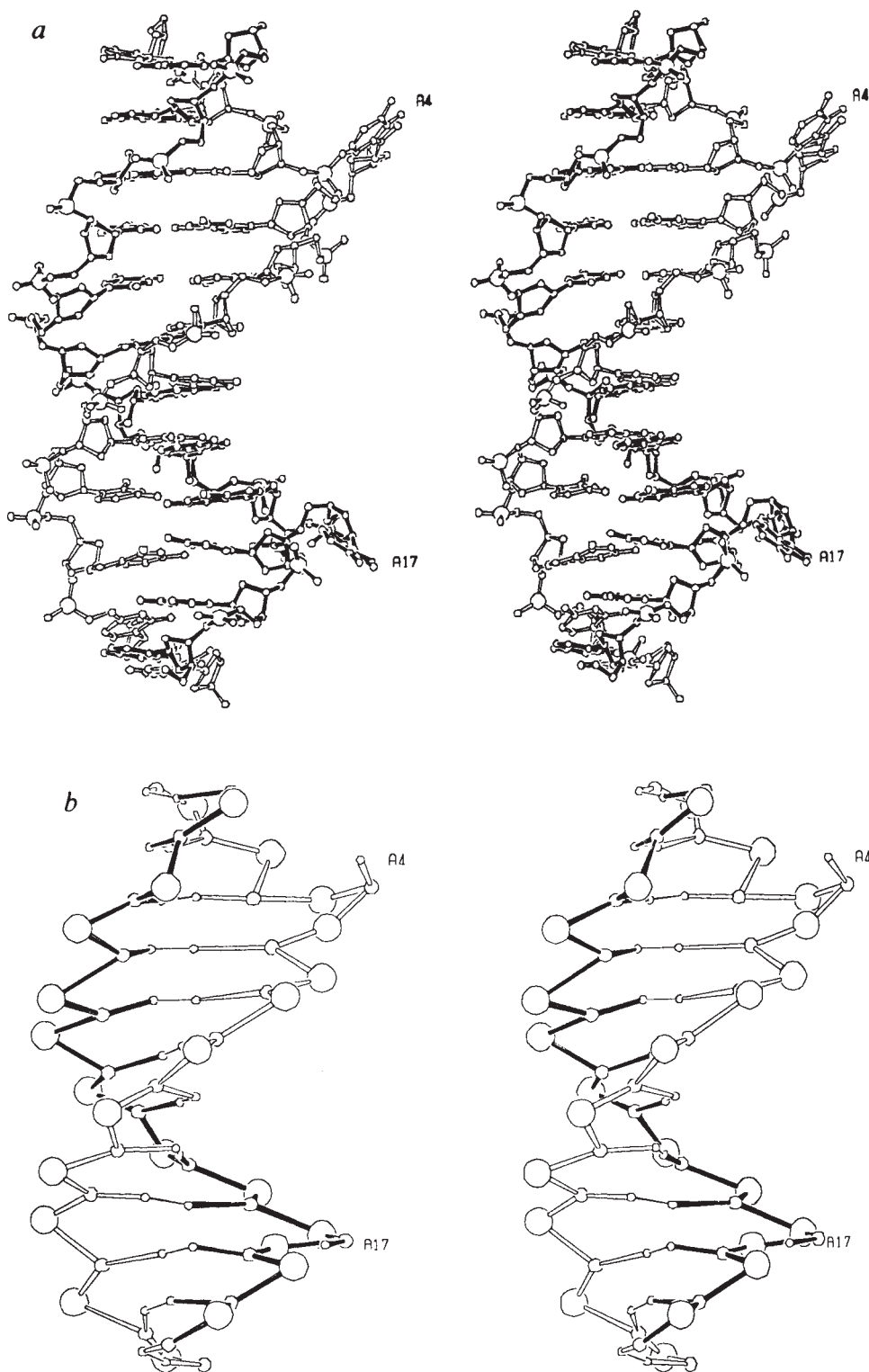


Fig. 1 Schematic representations of possible secondary structures for a tridecamer of the sequence d(CGCAGAATTCGCG)₂. a, An intact duplex with five misaligned base pairs. b, A modified duplex with the unpaired adenosines bulging out. c, A modified duplex with the unpaired adenosines stacked in the helix.

† Permanent address: Department of Chemistry, University of California, Davis, California 95616, USA.

§ To whom correspondence should be addressed.

Fig. 2 Stereo diagrams of the tridecamer $d(\text{CGCAGAATTCGCG})_2$ structure showing the extra adenosines bulging out. *a*, Ball and stick model with residues 1–13 in white and residues 14–26 in black. *b*, A schematic representation of the structure in the same view where the sugar-phosphate backbone is represented by phosphorous atoms (large spheres) linked to C1' atoms of the sugars (medium-sized spheres), and the bases by links from the C1' atoms to the N1 atoms in purines or to the N3 atoms in pyrimidines (small spheres). The base-pair interactions are drawn as thin lines. The overall conformation is close to that of B-DNA. The structure was solved via the molecular replacement technique, using the program ULTIMA⁹. A B-DNA dodecamer duplex (with the two extra adenosines omitted) was used as a search model. Initial rigid-body refinement led to an *R*-factor of 38% for the 25–6 Å data. The structure was refined in stages by the constrained restrained least-squares (CORELS) program¹⁹, first as a rigid-body, then as separate base pairs, and finally as individual phosphate groups and nucleosides, using only group thermal parameters²⁰. After the *R*-factor reached ~25% for 10–3 Å data, a difference Fourier map was calculated based on this refined dodecamer which showed clearly the presence of electron density at sites where the two omitted nucleotides might be. The backbones of these two additional residues, consisting of the phosphates and sugars, were initially fitted on a Vector General computer graphics display with the program FRODO²¹. After additional cycles of refinement and difference Fourier maps, the two adenine bases could be seen. Further refinement with the addition of 50 solvent molecules, using the Hendrickson-Konnert refinement program²² as modified by Westhof for nucleic acids²³, led to a current *R*-factor of 15.0% for 10.0–2.8 Å data for F_{obs} greater than 2σ (r.m.s. deviation of bond length from ideality is 0.01 Å). A detailed description of the structure solution will be given elsewhere.



bulge out from the double helix in quite different ways (Fig. 2). A4 is in the *anti*-conformation, extending out from the backbone and protruding into the minor groove. It appears to be stabilized in part by hydrogen bonding through a water molecule to an oxygen atom of phosphate 12 of a symmetrically-related tridecamer ($\frac{1}{2} - x, \frac{1}{2} + y, -z$). A17 is in a *syn*-conformation, lying along the backbone with its adenine base close to phosphate 16. The base pairs flanking the extra adenosines are stacked on top of each other.

Indications of the existence of other looped-out structures come from solution NMR studies of related sequences, including $d(\text{CAAACAAAG}) \cdot d(\text{CTTTTTTG})$, which contains an unpaired cytosine¹³, and $d(\text{CGCTGAGCTCGCG})_2$, which contains unpaired thymines⁴. In the case of the tridecamer $d(\text{CGCAGAATTCGCG})_2$ two conformations are evidently possible: one with the unpaired adenosines stacked into the duplex as found in solution, and one with the looped-out base as observed here in the crystal. Preliminary refinement of the

Fig. 3 A difference Fourier ($F_{\text{obs}} - F_{\text{calc}}$) map superimposed on a stereo drawing of the region of one of the extra adenosine nucleotides (A17). The entire A17 nucleotide was omitted in the computation of the calculated structure factors. Its density can be seen clearly in the map (lowest contour level at 2σ).

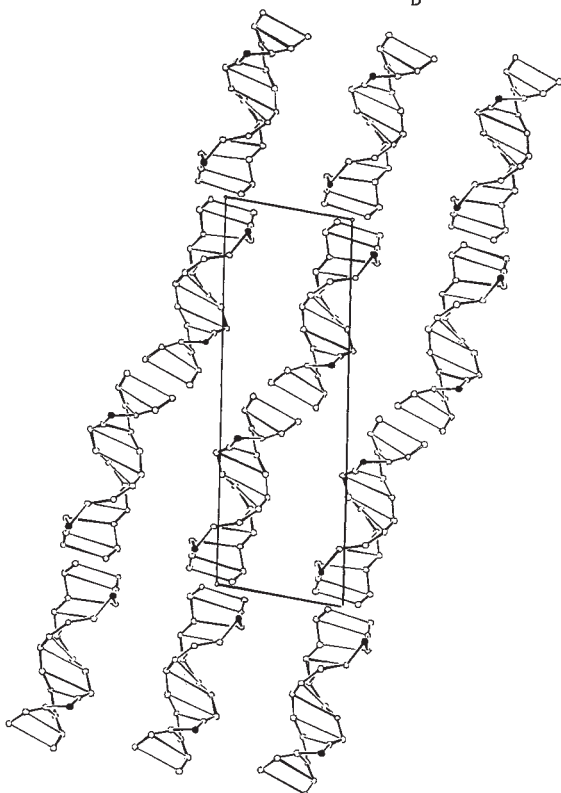
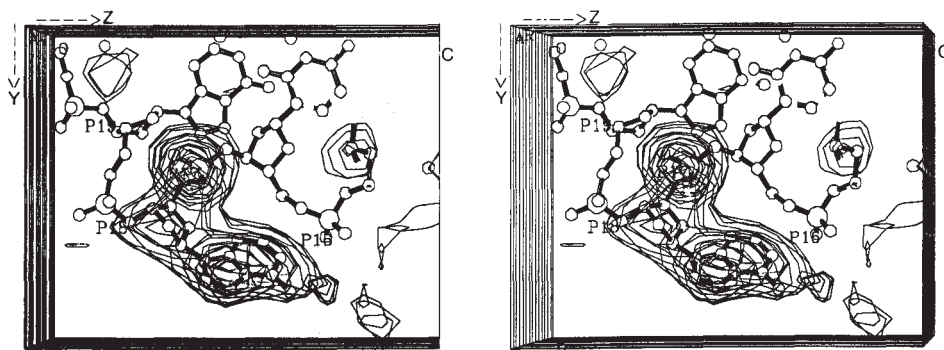


Fig. 4 A packing diagram of the DNA tridecamer structure showing one layer of molecules as viewed down the crystallographic b axis. Each strand is represented by lines connecting the $C1'$ atoms, and the base pairs are drawn as straight lines connecting the $C1'$ atoms on opposite strands. The two extra adenosine nucleotides are shown as filled circles. The packing in the crystal mimics a continuous DNA polymer with stacking interactions between adjacent duplexes. The rotation between the juxtaposed base pairs of neighboring duplexes differs from that of regular B-DNA, however, (-85° across the twofold axis at the origin and 39° across the twofold axis at $\frac{1}{2}, 0, \frac{1}{2}$).

crystal structure of a related 15-mer $d(\text{CGCGAAATTT-ACGCG})_2$ shows that, as in the tridecamer, the unpaired bases loop-out from the duplex¹⁴, although the packing scheme is different, as was the temperature of data collection. As for the tridecamer, however, two-dimensional NMR solution studies of the 15-mer indicate that these extra purines are inserted¹⁵. These studies clearly demonstrate the flexibility of the DNA molecule and suggest that even weak interactions, with other DNA molecules or proteins, could easily shift the conformation between two very different states. The salt-induced B-to-Z transition^{16,17} is another example of such a change.

The manner in which the tridecamer packs in this crystal simulates a long DNA polymer as the molecules stack lengthwise, although in alternating directions (Fig. 4). If the helix were disrupted, for example stretched or bent as in the structures derived by distance geometry calculations on two-dimensional

NMR data of $d(\text{CGCAGAGCTCGCG})_2$ (ref. 6) and by energy minimization for the tridecamer¹⁸, a B-DNA 'polymer-like' packing arrangement would probably not be possible and the resemblance to a long DNA molecule would be lost. In solution, where oligonucleotide molecules are well separated by intervening solvent, a DNA fragment of this size could accommodate an extra nucleotide either looped-out or inserted into the duplex. In fact, the duplex $d(\text{CGCTGAGCTCGCG})_2$ exhibits both conformations in solution, as observed by NMR studies at different temperatures⁴.

The three-dimensional structure reported here supports the slip-mispairing model proposed by Streisinger¹. It implies that certain DNA molecules can easily accommodate additional looped-out nucleotides in one strand, with minimal disruption of the overall duplex structure. This may be a clue to the observed tendency of certain DNA molecules to undergo frame-shift mutations. It is conceivable that the enzymes involved in DNA replication may be insensitive to a looped-out base in the vicinity of the replication fork, as the integrity of the local three-dimensional structure of the duplex is maintained. It will be especially intriguing to correlate the observed frequencies of frame-shift mutations in various DNA sequences with possible differences in the way the additional nucleotides are accommodated by either insertion or looping out from the double helix, and how the cellular repair mechanisms recognize and cope with these types of aberrant structures.

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Crystal structure of 15-mer DNA duplex containing unpaired bases

Maria Miller*, Robert W. Harrison*,
Alexander Wlodawer*, Ettore Appella†
& Joel L. Sussman‡

* Crystallography Laboratory, NCI-Frederick Cancer Research Facility, BRI-Basic Research Program, Box B, Frederick, Maryland 21701, USA

† Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892, USA

‡ Department of Structural Chemistry, Weizmann Institute of Science, Rehovot, 76100, Israel

Errors during DNA replication or repair can lead to the presence of unpaired or inserted bases in the double helix, as well as to mismatched base pairs. So far only structures of the latter type have been characterized by X-ray crystallography. We report here a 3-Å crystal structure of DNA 15-mer d(CGCGAAATTACGCG), which forms a duplex with two unpaired adenine residues looped outside the B-type helix. This arrangement is in disagreement with the nuclear magnetic resonance spectroscopy results for the same 15-mer in solution, indicating polymorphic nature of the structure adopted by this sequence.

The sequence of this DNA 15-mer contains a hot spot for purine deletion. Repetitive sequences of DNA can misalign during replication causing addition or deletion mutations, as was demonstrated in the case of bacteriophage T4¹. In T4, 12% of spontaneous one base pair deletions occur in nonrepeated sequences². As reported recently³, single base deletion mutations opposite a purine, flanked by a TT on the 5' side and a CG-rich sequence on the 3' side, frequently occur during *in vitro* polymerization with the large fragment of DNA polymerase I. The high frequency of this type of frameshift and its sequence specificity suggest a different mechanism than primer slippage. One possibility is the existence of an error-prone interaction between the TTPu sequence and the DNA binding site of the polymerase³.

Synthesis and crystallization conditions for the 15-mer have been reported previously⁴. The space group is *I*222 with *a* = 37.0 Å, *b* = 53.7 Å and *c* = 101.6 Å. X-ray diffraction data were collected with a Nicolet Imaging Proportion Counter, which is

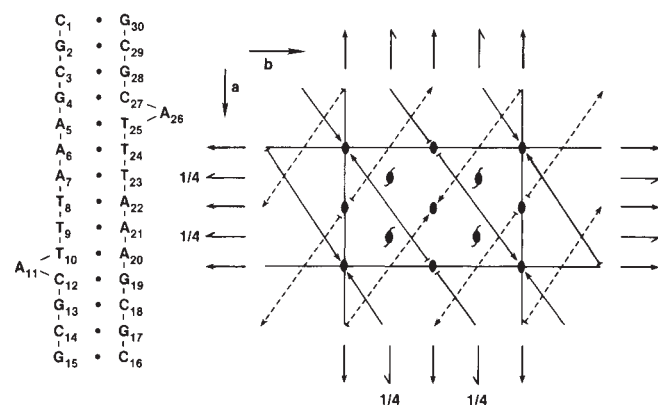


Fig. 1 Numbered sequence and the projection down the *z* axis of two consecutive layers of helices (represented by arrows), showing arrangement of the molecules in the *I*222 lattice. The monomers pack into long rods permitting base stacking interactions of the ends, but, because they alternate in direction, the rods are not simply continuous helices. In addition, they associate side by side in alternating directions to form sheets in the *x, y* plane. Helices from each consecutive sheet cross the next at an angle of 72° in the vicinity of the insertion.

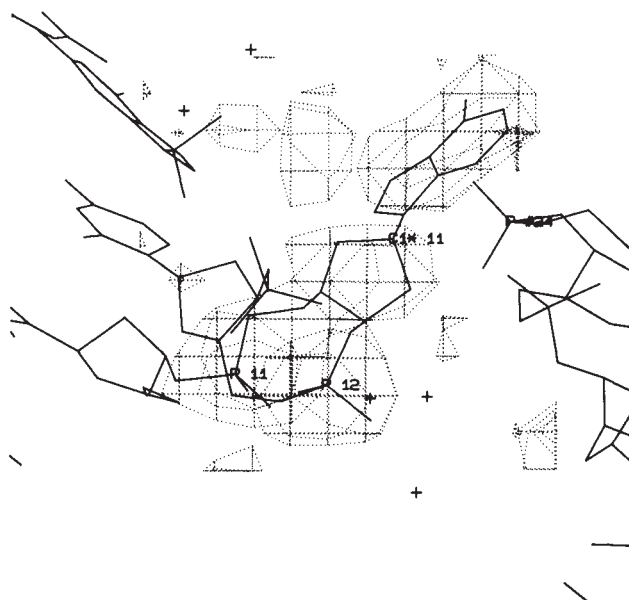


Fig. 2 View down the *z* axis of the $F_o - F_c$ Fourier map showing the electron density contoured at 2σ level around adenosine 11. The map was calculated after removing this residue from the model used for calculating phases. The coordinates are from the final model showing contacts with P24 of the symmetry related molecule.

an electronic area detector⁵. The configuration of this instrument has been described in detail elsewhere⁶. The area detector chamber was mounted 10 cm from the crystal and the carriage angle was set at 7°. The integration of reflection intensities was performed with the XENGEN program system⁵. Crystals were kept at 4 °C during X-ray exposure using an FTS XR-85-1 gas-stream crystal cooler. Data were collected on two crystals and included 18,446 reflections. Out of a possible 2,202 unique reflections to 3-Å resolution, 1,943 has significant intensity, with $I > 1.5\sigma(I)$. The weighted scaling *R* factor on intensity⁵ was 0.053, but the intensities were weak at high resolution. We were not successful in making any heavy atom derivatives but the phases were

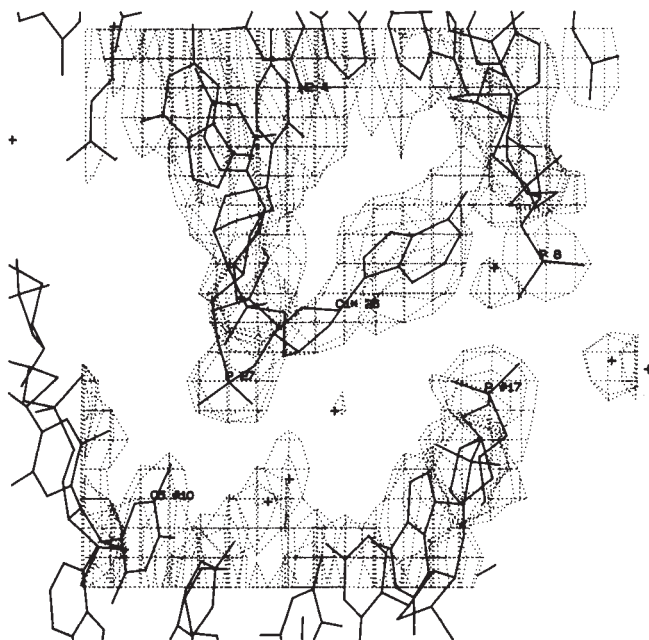


Fig. 3 View down the *y* axis of the $2F_o - F_c$ map, showing density for adenosine 26 in the minor groove of the helix in the vicinity of a symmetry mate, but too far to form direct intermolecular contacts.

obtained by applying a combination of direct methods and minimization of cross entropy, as described elsewhere⁷.

The first electron density map clearly showed the location and orientation of the DNA molecule⁷. The ~ 3.35 -Å base pair separation and the location of the major and minor grooves were also visible. As expected from the analysis of the diffraction patterns recorded on precession photographs, the helix axis lies in the x, y plane⁴, along the line connecting the set of non-equivalent twofolds perpendicular to this plane (Fig. 1). Simple packing considerations suggested 14 base pair duplexes packed as infinite cylinders between the two crystallographic twofolds. This would imply that the two unpaired adenine residues were looped out.

The refinement of such a model was performed with the program NUCLSQ, which is a modification by Westhof⁸ of the protein refinement program PROLSQ of Hendrickson⁹. NUCLSQ restrains distances and general geometry including hydrogen bonds between complementary bases. No restraints were applied to sugar-phosphate torsion angles. The crystallographic R -factor for the final model is 26.3% with a single overall thermal factor (r.m.s. deviation of bond lengths from ideality was 0.026 Å). The R factor may be reduced to 24% and the geometry residuals decreased to 0.02 Å with the introduction of individual thermal factors, but these are not meaningful at this resolution. The electron density maps, both $F_0 - F_c$ phased with a model excluding the extra adenosines (Fig. 2) and $2F_0 - F_c$ (Fig. 3), are in agreement with the structure. Finally, the packing of the molecules into the unit cell is very tight. It is difficult to move the model more than 1 or 2 Å in any direction without making an unacceptable number of bad contacts between the molecule and its crystallographic symmetry mates.

The two other possible models for this 15-mer—one with the unpaired bases inserted into the helix and the other with a 'head-to-tail' aligned duplex with three mismatches—would result in longer helices, 16 and 15 base pairs long respectively (also see Fig. 1 of ref. 10 for similar models for the tridecamer). This would require a staggered packing. It must be stressed that it is possible to make versions of these models with most of the atoms in nearly the same positions (or equivalent ones). These putative models were tested and would require either unwrapping or unwinding of one or both ends of the helix to fit into the unit cell. This results in poor geometry and many bad contacts with the symmetry mates. Furthermore, in neither case did either the $2F_0 - F_c$ or nucleotide deleted maps agree completely with the structure.

The 15-mer duplex adopts an overall B-DNA structure (Fig. 4). The two unpaired adenines are looped out but, despite the palindromic sequence, each nucleotide is oriented differently relative to the double helix. This is undoubtedly due to the crystal packing forces, as the two adenines are in nonequivalent crystallographic environments (Fig. 1). Adenine 11 makes contacts with the phosphate of residue 24 of a symmetry-mate helix situated antiparallel in the x, y plane (Fig. 2), while adenine 26 lies in between helices from adjacent sheets. Part of the sugar-phosphate backbone penetrates slightly into the major groove of a helix from the consecutive sheet. For this reason adenine 26 is pushed into the minor groove of its parent double helix (Fig. 3). In the crystal structure of the DNA tridecamer¹⁰, which crystallizes in space group $C2$, the unpaired adenosines are also looped outside but have a different local structure from that observed here.

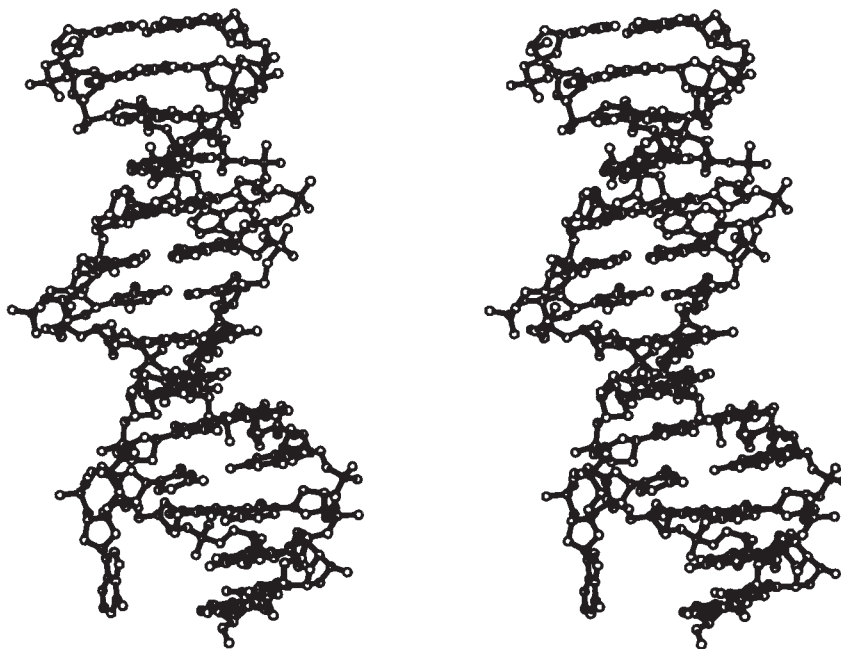


Fig. 4 Stereoview of the skeletal model of the duplex, showing adenosine 11 outside the double helix. Unpaired adenosine 26 is also visible in the minor groove, about one third of the molecule away from the top.

Two-dimensional nuclear magnetic resonance spectroscopy (NMR) studies of this DNA oligomer demonstrated that in solution the inserted bases are stacked within the helix¹¹. When this is the case the molecules are probably kinked at the insert site, forming S-shaped structures. The difference between the conformation observed by NMR in solution, and the straight double helix with bases looped out as observed in crystalline state, may depend on the degree of hydration and the presence of spermine. A similar discrepancy between conformation in solution and in solid state was observed in the case of the DNA tridecamer d(CGCGAATTCGCG)¹⁰. The correlation between the positions of the unpaired bases outside the helix and crystal packing clearly indicate their flexibility depending on the environment. In the case of biosynthesis, if there is a stronger interaction between the adenine and the polymerase than between the adenine and its flanking bases, then it is possible that the adenine will be in the wrong position during polymerization. When this is the case the thymidine opposite may not be incorporated and a single base deletion mutation will be formed. As suggested by the structure presented here, the resulting double helix is very similar to the canonical B-DNA¹² so that the polymerase may continue despite an error.

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HPLC in nucleic acids research

J. A. Thompson and R. D. Wells

The separation capabilities of ion exchange, when coupled with the principles of reversed-phase fractionation, yields an advanced technology for the manipulation of nucleic acids.

THE extraordinary resolving power of gel electrophoresis has allowed it to virtually replace older physical methods, such as ultracentrifugation, for molecular weight analyses of nucleic acids. But high performance liquid chromatography (HPLC) has become the method of choice for the separation and purification of these molecules. Modern advances in HPLC have achieved resolutions of nucleic acids that approximate or surpass those obtained by gel electrophoresis^{1,5}.

Nevertheless, gel electrophoresis techniques (both agarose and polyacrylamide) probably have been the most widely used non-HPLC methods for the isolation and analysis of nucleic acids. The popularity of gel electrophoresis arises from its low costs, and its capability to process simultaneously a relatively large number of different samples. The disadvantages of gel electrophoresis are that it is relatively time-consuming and labour-intensive, and it recovers only small amounts of recoverable chemically or biologically active nucleic acids. Furthermore, gel electrophoresis does not lend itself to either automation, large-scale (milligram) separations, or the purification of species which are similar in molecular weight but which differ in base composition. The more modern techniques of HPLC can more adequately address the requirements for speed, scale-up and automation of nucleic acid purification.

Chromatography resins

At the heart of all HPLC applications is the chromatography resin. Classical HPLC included the use of celluloses, dextrans and agarose-based supports which were originally developed for applications in protein purification. The lack of applications for nucleic acids and certain other disadvantages^{1,3,5} prevented the widespread adoption of HPLC techniques. However, parallel advances in the development of both silica- and polymer-based HPLC resins have found applications in the analytical and preparative separation and purification of nucleic acids.

The development of chemically resistant polymer matrices and porous micro-particulate supports such as silica, the binding of specific functional groups to these supports, and improved packing techniques have increased the efficiency of chromatographic columns in nucleic acid separations⁶⁻¹⁰. However, simply chemically bonding classical functional groups to the new support matrices does

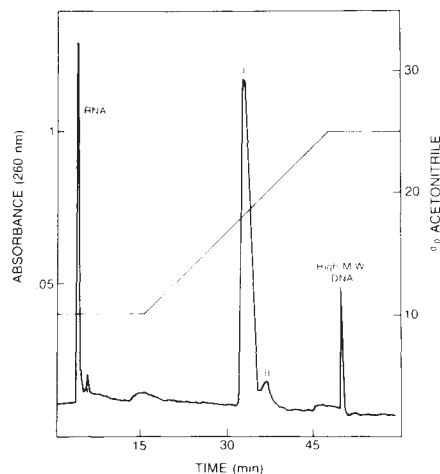


Fig. 1 HPLC-PICS purification of plasmid DNA from *Escherichia coli*. Eluted plasmid DNA contained ~ 90% supercoiled (I) and 10% nonsupercoiled (II) molecules².

not provide an easy transfer of HPLC technology to nucleic acids research.

Although nucleic acids consist of nucleotides with defined chemical properties, their chromatographic behaviours differ significantly as the chain length of the polymer increases. Chemical studies have revealed that secondary and tertiary structures profoundly affect the physical characteristics and chemical reactions of nucleic acid molecules. While biochemical separations of peptides, proteins and other low-molecular weight compounds have been aided by varying the particle and pore size of the various supports, the HPLC supports often contributed to problems of non-specific binding, poor resolution, poor recovery and shearing of nucleic acids.

Some of the earliest successful chromatography systems for nucleic acids employed an inert solid support coated with a film of a water-immiscible extractant which conferred anion exchange properties onto the solid support (RPC-5, RPC-5 Analog, NACS), but several difficulties encountered with these original systems hindered their widespread adoption. Most of the difficulties subsequently were corrected, except for leaching of the ion-exchanger.

In these early anion exchange systems, the functional organic layer (quaternary amine) was coated rather than covalently attached to the inert support, so this layer was gradually released with continued use, reducing the effective life of the column. Covalent attachment of the ion-

exchanger to silica-based resins only partially solved these chromatographic problems. Use of these ion-exchange chromatography resins still requires the elution of the nucleic acids by gradients of increasing salt concentration, usually in the presence of solvent modifiers such as urea or formamide. This process requires time-consuming removal of the reagents from both collected nucleic acid samples and the HPLC instrumentation.

Paired-ion HPLC

Current research has focused on integrating the principle strengths of previous methods while eliminating their observed weaknesses. The paired-ion chromatography system (PICS) has been developed to combine the power of ion-exchange and reversed-phase chromatography for the purification of nucleic acids. With PICS, both analytical and preparative separations can be achieved using the established principles of reversed-phase ion-pair chromatography¹¹, which resolves nucleic acids based on their available charge density — a function of both molecular weight and nucleic acid structure.

The phase system consists of a chemically modified, inert, non-porous polymer support (reversed-phase) and the use of a hydroorganic eluent containing an ion-pair reagent (triethylamine acetate). The system is simple to use and provides flexibility and selectivity. It does not depend upon coating or covalently attaching the ion-exchanger to the solid support: the exchange ligand (triethylamine) forms an ion-pair with the nucleic acid in the mobile phase, and the resulting nucleic acid-ligand ion-pair complex is strongly retained in the column via hydrophobic interaction with the inert, solid support. Once retained, the nucleic acid-ligand ion-pair complexes can be eluted with organic solvents at concentrations characteristic of the specific nucleic acid species.

The success of PICS in the HPLC of nucleic acids can be attributed to many factors. The inert polymer matrix has a high recovery rate (greater than 90 per cent), and high capacity because of the irregular shape and discrete size of its particles. The lack of pores in the matrix eliminates steric exclusion principles, and the covalently derivatized polymer surface allows the mobile phase to be manipulated to create desired conditions. The volatile solvents used also have less chemical resistance than the aqueous salt solutions required in other systems.

PICS can be used for the column purification of plasmid DNA (Fig. 1)², DNA restriction fragments³ and synthetic oligonucleotides⁵. The resolved nucleic acids are recovered in volatile solvents, which are easily removed by lyophilization, rotary evaporation, ethanol precipitation, dialysis or a combination of procedures.

Batch processes

The ease of workup required for PICS allows it to be adapted for an even more powerful application: batch processing. All plasmid DNA molecules, regardless of molecular weight, elute from a PICS column at the same concentration of acetonitrile, suggesting that the available charge density of the supercoiled structure determines its binding and elution conditions. This observation makes PICS amenable to autosampling for repetitive HPLC applications, but even more importantly means that it can be used in syringe methods and spin columns for batch purifications of nucleic acids.

These "minicolumn" methods are invaluable for manipulating small quantities (1 pg to 20 µg) of either DNA or RNA, which are easily recovered by lyophilization. Neither proteins nor free nucleotides will bind to the inert solid support, making the PICS cartridges useful for removing unincorporated radioactive nucleotides, "cleaning up" nucleic acid reactions involving enzymes, desalting and concentrating dilute nucleic acid solutions, extracting nucleic acids from clinical samples and purifying nucleic acids following gel electrophoresis. The biological activity of these recovered nucleic acids has consistently remained the same as or better than nucleic acids purified by other methods^{1,5}.

Columns for performing HPLC-PICS are now commercially available under the trademark Polypore PICS (Applied Biosystems, Inc., Foster City, California). Both disposable syringe cartridges and spin columns for performing batch-PICS are now commercially available under the trademark Aladex (Berwick Life Science Division, Warminster, Pennsylvania). □

John A. Thompson and Robert D. Wells are cofounders of Alatech Associates, Inc. and are at the Department of Biochemistry, University of Alabama at Birmingham, Schools of Medicine and Dentistry, Birmingham, Alabama 35294, USA. For more information, fill in reader service number 100.

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Biochemistry briefs

Next week in Prague, Czechoslovakia, the 14th International Congress of Biochemistry will convene. Products on display in the exhibit hall will cover the gamut from analytical instruments to production-scale equipment.

AMERSHAM exhibiting in booth 40, has launched an **IP₃ assay** which should enable researchers to measure levels of this second messenger and to study its function in metabolism, secretion, cell growth, contraction, phototransduction, the nervous system, and oncogene action and cell proliferation (*Reader Service No. 101*). Amersham says the D-myo-inositol 1,4,5-trisphosphate ³H assay system is a sensitive and convenient kit for the measurement of inositol 1,4,5-trisphosphate (IP₃) levels in large numbers of samples. The company reports that the assay is specific for the 1,4,5-isomer of IP₃; the cross-reactivity rate of the binding protein used is less than 10 per cent for inositol 1,3,4,5-tetrakisphosphate and less than 1 per cent for other inositol phosphates. Each kit contains sufficient material for 100 assay tubes, which allows the construction of one standard curve and the determination of 39 unknowns in duplicate. Amersham says the assay can measure less than 0.2 pmol of IP₃ over a working range of 0.19-25 pmol per tube, and that the test takes 3-4 hours, with hands-on time of only 1 hour.

Beckman Instruments, exhibiting in booth 4, has recently introduced three new **ultracentrifuge rotors** for specialized applications in bioresearch (*Reader Service No. 102*). Two of the new rotors — the \$8,100 (US) model VC53 and the \$6,900 (US) model VAC50 — are made of light-weight materials for fast handling. The third rotor, the \$7,450 (US) type 50.4 fixed angle rotor, is made entirely of titanium and is especially suited for lipoprotein separations. The model VC53 composite rotor has titanium tube inserts and hollow construction: at only half the weight of standard titanium rotors, Beckman says it is twice as fast. The model VAC50 rotor's composite construction has an aluminium core for reduced weight, but it holds 25

per cent more volume than similar titanium rotors. The Beckman type 50.4 titanium rotor spins at 50,000 r.p.m., with two tiers holding 44 6.5-ml tubes at a fixed 20° angle.

Gel gems

Pharmacia LKB Biotechnology has just announced the availability of three new **precast electrophoresis gels** for use with its PhastSystem (*Reader Service No. 103*). The PhastGel homogeneous media come in three different polyacrylamide concentrations: PhastGel homogeneous 7.5, PhastGel homogeneous 12.5 and Phast-



Precast gels from Pharmacia.

Gel homogeneous 20. Pharmacia says the use of its PhastGel media with the PhastSystem will allow researchers to more closely examine molecular weight regions of interest. The company recommends PhastGel 7.5 for the separation of larger proteins and for rapid separation of proteins, PhastGel 12.5 for medium molecular weight separations, and PhastGel 20 for the separation of smaller proteins and proteins of similar molecular weight. Pharmacia will be exhibiting in booth 3.

Just about every reagent needed for electrophoresis is contained in the new **electrophoresis source book** released from ICN Biomedicals, Inc. (*Reader Service No. 104*). The free book contains a complete listing of ICN's ultra-pure chemicals and reagents for electrophoresis work, including products for polyacrylamide and agarose electrophoresis, isoelectric focusing, protein blotting and transfers, and affinity purification of proteins. The book is organized into sections, and lists products according to their applications: there are chapters on cross-linking reagents, buffers, stains and dyes. Also featured are electrophoresis kits, premixed buffers and specialty reagents. ICN will be in booth 1.



Beckman's centrifuge rotor roundup.

Chromatography hardware

A new **photodiode array-based HPLC** with Waters Powerline HPLC control, integration and networking capability has



Photodiode array-based HPLC from Waters.

been introduced by the Waters Chromatography Division of Millipore (*Reader Service No. 105*). The Waters Powerline PDA system features the Waters 990+ photodiode array detector and 600E gradient module with optional automated sample processing. Waters says the 990+ PDA detector provides full spectral acquisition and monitoring from 190 to 800 nm with post-run processing routines which perform contour plot, 3-dimensional display, quantitation, and overlay functions for up to six normalized spectra. A special spectral library routine allows newly acquired and stored spectra to be compared. The gradient module provides isocratic or gradient delivery of up to four solvents with flow rates from 0.01 to 45 ml min⁻¹. Millipore is in booth 30.

Amicon, in booth 31, has introduced a computer-automated chromatograph for aqueous **low-pressure process chromatography** — the K-Prime 400 (*Reader Service No. 106*). Amicon says its new



Amicon's setup for process chromatography.

member of the K-Prime series of chromatography systems provides software control of process methods and enables a PC-based network of automated chromatographs. The system allows process validation, report generation and precise computer control of chromatographic function, and accepts columns from 70 mm to 2 m. The \$60,000-\$70,000 (US) K-400 system consists of a microprocessor-based control unit, process chromatograph and a PC interface. The addition of a PC with

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networking software permits remote control and monitoring of multiple systems.

A **high-pressure pump** with built-in software that controls analytical and preparative HPLC systems has been introduced by Gilson Medical Electronics (*Reader Service No. 107*). The new model 305 pump communicates with as many as four other Gilson pumps to generate mobile phase gradients at flow rates of between 0.5 µl min⁻¹ and 200 ml min⁻¹. The pump can be connected to a computer which runs Gilson's control and data analysis package for more sophisticated system automation, and a 10 mV analogue input can be used to permit the pump to function as an auxiliary data acquisition device. As many as 10 sets of control



Gilson's newest pump for HPLC.

parameters can be stored in non-volatile memory. The \$3,200 (US) model 305 pump is compatible with all Gilson pump heads, including a 200 ml min⁻¹ titanium head designed for preparative biopolymer separations and inorganic analyses. Gilson will be exhibiting selections from its line of chromatography products in booth 6. □

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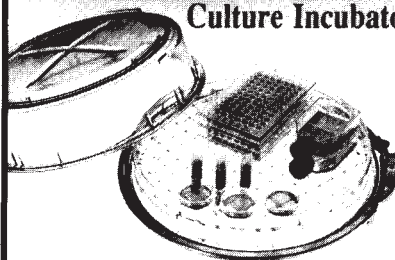
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Graduate crisis looms in the 1990s

Richard Pearson

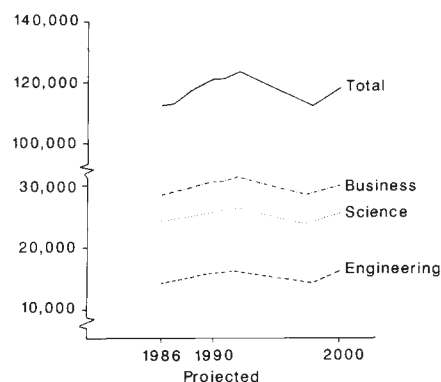
The demand for graduates will continue to increase over the next ten years, and employers must change their ways if they are to attract their share of scarce skills.

WITH the economy in its seventh year of expansion, shortages of graduates are becoming more widespread, and at the end of last year half the graduate recruiters had unfilled vacancies. This year, the proportion is likely to be higher still as demand grows by 5 per cent or more, while the numbers graduating lag behind. Demand for graduates is booming as employers see them as the key to increased effectiveness and competitiveness. They are increasingly being sought not only in the traditional areas of research, management and administration and professions such as law and accountancy, but also in new growth sectors such as retailing, tourism and the fast food chains.

Looking ahead to the 1990s, the 35 per cent decline in the number of school leavers is likely to give another twist to the growing problem of shortages, to the benefit of the majority of graduates. This year, starting salaries are likely to average over £9,000, or nearly 80 per cent of average male earnings, well up from the 67 per cent in 1982. The 1980s also saw a widening of salary differentials, with the few top jobs in the city offering £16,000 or more and some oil companies and consultancies offering over £12,000. Two years ago, competition for the best graduates had become so intense in the City of London that "golden hellos" and signing-on-fees of £2,000 were being offered, although they appear less prevalent now. But at the other end of the scale some jobs are offering only £7,500.

The past decade has been one of turbulence and change for graduates. It was ushered in by a major recession and rising unemployment. The numbers graduating continued to grow, driven by the expansion of the polytechnics and colleges which more than compensated for the cut-backs in universities. As the economy moved out of recession, demand started to grow rapidly, particularly for financial work, where chartered accountants are now recruiting 1 in 10 of those seeking jobs, and the retail sector. Demand has also gone up in more traditional areas such as engineering and government. The only black spot is the near 50 per cent fall in the intake to teaching which has exacerbated teacher shortages. In total, 30 per cent more graduates entered employment last year than in the recession year of 1981. There have been hiccups — in 1986, the downturn in the electronics and computer industry led to sharp reductions in gradu-

ate intakes. Major recruiters are now to be found in all sectors of the economy; the two largest graduate recruiters are now GEC/Marconi, the electronics group, and Peat, Marwick, McLintock, the accountants, each of which is seeking more than 1,000 graduates this year. British Airways, Courtaulds, ICI and Sainsbury are all seeking more than 200, and McDonalds,



Projected first degree graduate output 1986-2000. Source: IMS/DES.

the fast food chain, is seeking over 150.

Not all graduates have benefited from this boom. The unemployment rate six months after graduation last year was over 7 per cent, compared with 5 per cent of a smaller group in the last boom year of 1979. A significant number of other graduates are in temporary jobs while they try to find jobs more appropriate to their skills. Poor personal qualities and locational constraints are seen as two of the major barriers to good jobs.

The numbers graduating are expected to continue to increase from about 115,000 this year to about 124,000 in 1992, an increase of 10 per cent, but the number of university mathematics and physics graduates, for example, is expected to fall next year. The most significant factor affecting the number of graduates beyond 1992 is the 25 per cent fall over the next six years in the number of 18-year-olds, the traditional entry group to higher education. As most entrants come from middle-class families, whose birth rates have fallen more slowly than the average (see *Nature* 329, 470; 1987), the fall in applications is not expected to be as great, and it is hoped to increase the intake from non-traditional groups such as mature entrants, ethnic minorities and those without A-level qualifications. Output in 1992 is likely to be up to 10 per cent higher

than in 1986, but by 1998 should fall back to this 1986 figure before starting to rise again (see figure). These figures are based on the government's hope that nearly one in five of the relevant age group will go into higher education, up from 14 per cent today, counteracting the demographic effect. This is a challenging figure by UK standards and one which could become more difficult if student loans were to be introduced. The apparent swing away from engineering and technology and the sciences, as measured by university applications, when combined with the effect of demography, is likely to mean that the output of graduates in science and engineering in the mid-1990s will be little better than today.

Over the past five years, demand for graduates has been growing at an annual rate of nearly 5 per cent. Demand in the 1990s will be affected not only by economic growth but also by technological and organizational factors. All the forecasts point to a growing need for graduate-level skills; the numbers of managers are expected to grow by 20 per cent, professionals by 17 per cent and scientists and engineers by 20 per cent over the decade to 1995. More graduates are also expected to go into small businesses and self-employment. If demand were to increase as it has over the past decade, intakes would be 20 per cent or more higher by 2000, and this would reflect a modest growth rate of only 1.5-2 per cent a year, well below the current rate of increase.

Even if it is not possible to forecast the precise level of demand, it seems likely, barring a major economic downturn, to exceed the level of output in the 1990s and competition among employers will intensify, especially if they continue to shun the mature graduate who will be forming an increasing proportion of the output. With the cost of graduates also likely to rise significantly, employers must start now to improve their use of scarce skills, to sharpen and widen their recruitment activities and to increase their support for all levels of the education system if they are to attract their share of scarce skills. For the individual, the return from three years in higher education is likely to continue to improve so long as a balance of academic and personal attributes is developed. □

Richard Pearson is at the Institute of Manpower Studies, Mantell Buildings, University of Sussex, Brighton BN1 9RF, UK.